Historic, Archive Document

Do not assume content reflects current scientific knowledge, policies, or practices.

1.9
P691Rp

LIBRARY
RECEIVED
MAR 15 1939
U. S. Department of Agriculture

1938

Second Annual Report

U. S. Regional Pasture Research Laboratory

State College, Pennsylvania

Division of Forage Crops and Diseases

Bureau of Plant Industry

and

The Agricultural Experiment Stations

of the

Northeastern States

Cooperating

[Washington, 1939]

Twenty-nine copies of this report were made and distributed as follows:

Three copies to the Division of Forage Crops and Diseases; one copy to each of the twelve Directors of the cooperating State Agricultural Experiment Stations in the Northeastern United States; one copy to the President of the Pennsylvania State College; one copy to the Director of each of the following State Agricultural Experiment Stations: Illinois, Indiana, Iowa, Kentucky, Michigan, Minnesota, Missouri, Ohio, and Wisconsin; and the remaining four copies to the U. S. Regional Pasture Research Laboratory.

THE LABORATORY STAFF

R. J. Garber . . . . Director

Alice M. Atkinson . . . . Secretary

Project Leaders

J. T. Sullivan . . . . Physiologist

W. M. Myers . . . . Associate Geneticist

V. G. Sprague . . . . Associate Agronomist

R. R. Robinson . . . . Associate Agronomist

S. S. Atwood . . . . Assistant Geneticist

W. H. Brittingham . . . . Assistant Cytologist

S. J. P. Chilton . . . . Assistant Pathologist

Technical Assistants

J. G. Conti . . . . Biochemistry

Helen D. Hill . . . . Cytogenetics

G. H. Ahlgren . . . . Genetics and Physiology

Greenhouse and Nursery Foreman

Ralph Kern

COLLABORATORS

B. A. Brown, Connecticut

G. L. Schuster, Delaware

J. A. Chucka, Maine

J. E. Metzger, Maryland

W. B. Colby, Massachusetts

F. S. Prince, New Hampshire

C. B. Bender, New Jersey

C. E. F. Guterman, New York,

S. I. Bechdel, Pennsylvania

G. E. Gilbert, Rhode Island

G. P. Burns, Vermont

G. G. Pohlman, West Virginia

TABLE OF CONTENTS

RESEARCH AT THE LABORATORY (continued) Page

Page

RESEARCH AT THE LABORATORY (continued)

Appendix A

Appendix B

Appendix C

REPORT OF

THE UNITED STATES REGIONAL PASTURE RESEARCH LABORATORY

FOR THE CALENDAR YEAR,

JANUARY 1, 1938, to JANUARY 1, 1939.

INTRODUCTION

During 1938 definite progress was made in rounding out the research program at the U. S. Regional Pasture Research Laboratory. In conformity with the established policy of developing research at the Laboratory, not already adequately provided for in the Northeastern States, facilities for pathological investigations of pasture plants were established and some progress made in actual research. The investigations concerned with the cytogenetics and breeding, as well as those concerned with the chemical composition and physiology of pasture grasses and legumes, were carried forward much as outlined. In all of this work effort was made to develop the various phases of research along lines of regional, rather than of local, interest.

One of the purposes of establishing a regional Laboratory for pasture research in the Northeastern United States was that it serve as a focal point for coordinating and integrating fundamental pasture research in the Region. Pursuant to achieving this objective, two important meetings were held during the year under review, namely, the meeting of plant breeders interested in pasture improvement in the Northeast and the annual meeting of the collaborators.

This annual report in common with most annual reports of a similar nature contains preliminary information and tentative statements which may not be verified by subsequent experiments. For these reasons, viewpoints expressed and deductions drawn should not be regarded as final. It is probably unnecessary to point out that data and statements appearing in

the report are not for publication.

The appendixed contain detailed reports of the collaborators' meeting and the plant breeders' meeting, as well as a progress report, collected by the collaborator, for each State station collaborating with the Laboratory. This last feature is particularly valuable since it makes possible, within a single report, a comprehensive survey of the pasture research under way in the Region.

A more or less detailed description of the activities at the Laboratory follows.

CHANGES IN PERSONNEL

Last July a project leader in plant pathology, S. J. P. Chiltoh, who received his graduate training at the University of Minnesota, was added to the Laboratory Staff. J. G. Conti, an assistant in biochemistry, Mrs. Helen D. Hill, a technician in cytogenetics, and G. H. Ahlgren, a part-time assistant in the work with Sudan grass, were other additions to the staff during the year. With the increase in greenhouse and nursery work, it was necessary to augment considerably the corps of laborers. Fortunately for the continuity of the research under way no resignations among project leaders occurred. R. H. Burton, the former secretary of the Laboratory, resignsd to accept an appointment elsewhere and Mrs. Alice M. Atkinson was appointed in his place.

Owing to the fact that W. H. Pierre, collaborator from the West Virginia station, resigned to accept the chairmanship of the Department of Crops and Soils at Iowa State College, G. G. Pohlman, present head of the Department of Agronomy and Genetics, at the West Virginia Agricultural Experiment Station, was appointed in his place.

ADDITIONS TO BUILDINGS AND EQUIPMENT

The most important addition to the physical equipment made during the year was a new greenhouse, 122 feet long and 35 feet wide, divided into five sections and connected with one of the former greenhouses by a glazed passageway large enough to accommodate soil storage bins and potting benches.

Scientific equipment was purchased and installed in the plant pathology laboratory and some other important items, such as constant temperature ovens and a muffle furnace, were obtained for the other laboratories. Several compressors with attached motors were transferred to the Laboratory without cost except transportation. After reconditioning, two of the units were used to provide refrigeration for a "walk-in" freezing chamber.

The small power saw and planer and the drill press purchased during the year have been used in connection with constructing numerous items, such as tables and shelves for the rooms in the headhouse, additional plant benches in the greenhouses, shelving and insulation in the drying room, inoculating chambers for pathological investigations, and small tables mounted on casters for various uses in the laboratories and greenhouses. The concrete walks in the new greenhouse were constructed by permanent employees of the Laboratory. The three greenhouses were wired and provided with time switches for supplemental lighting at cost by the Department of Grounds and Buildings of the Pennsylvania State College.

An additional acre of land was made available for nursery purposes, bringing the total to about 16 acres.

ACTIVITY RELATING TO COORDINATION OF RESEARCH

It will be convenient to discuss the activities relating to the coordination and integration of pasture research in the twelve northeastern

States under three subheads, the meeting of the plant breeders, the meeting of the collaborators, and miscellany. Inasmuch as detailed reports of the two meetings are given in appendixes B and C, respectively, only the main objectives attained at the meetings, and a few other interesting facts, will be mentioned here.

Meeting of Plant Breeders in the Northeast

Following a suggestion made by the collaborators, a meeting of plant breeders interested in breeding pasture plants in the northeastern States was held in New York City, March 18 and 19, 1939. Nineteen delegates, comprising both State and Federal workers in the Region, attended the meeting.

The purpose of the meeting was to arrive at a mutual understanding of the functions of the Laboratory in a coordinated breeding program for the Region and to discuss problems and methods of attach. It was mutually agreed that the Laboratory, insofar as its own breeding research is concerned, should lay particular stress on the discovery of facts and principles and underlying breeding rather than to breed directly for improved strains, an activity which perhaps can be carried on more advantageously by the various State stations or by them in cooperation with the Laboratory. Topics which were discussed at the meeting follow: Characters to be sought in breeding pasture plants; possible value of exploring existing variations within species now growing in pastures of the Northeast; should attention be confined to the so-called permanent pasture plants; reproduction in the <u>Poas</u>; apomixis, its occurrence and significance; controlled pollination of pasture plants; self-, cross-, and male-sterility and their place in a breeding program; inbreeding and heterosis and their possible significance, particularly with reference to clover and grass

breeding; existing polyploids among pasture species; chromosome behavior and interspecific hybrids; induced polyploidy as a means of extending the range of hereditary variation; methods of breeding pasture plants with reference to certain species; and, seed problems in connection with pasture improvement through the use of improved strains. (See Appendix B.)

Meeting of Collaborators

The second annual meeting of the collaborators was held at State College, on September 7 and 8, with all cooperating stations represented except West Virginia and New Hampshire. Most of the meeting was given over to considering the progress of research at the Laboratory during the past year and present plans. At future meetings of the collaborators it is expected that relatively more time will be given to considering the State programs of pasture research. (See Appendix C.)

Miscellany

In order to help keep the pasture research workers in the Region mutually informed, the project outlines of the Laboratory's research were brought up to date and mailed to each of the twelve cooperating State stations and similarly revised project outlines were received at the Laboratory from the State stations. In this connection should also be mentioned the progress reports of the pasture research under way at the State stations in the Region submitted by the collaborators and produced in Appendix A. During the year one or more members of the Laboratory staff visited Connecticut, Massachusetts, Maryland, New Hampshire, New Jersey, New York, and West Virginia, to discuss with pasture research workers problems of mutual interest. Laboratory visitors for the same purpose included staff members from the Maryland, New Jersey, New York, and West Virginia agricultural experiment stations. These exchanges of visits,

which were in addition to those incident to the regularly scheduled meetings, help to promote a mutual understanding of one another's professional problems and hence facilitate cooperative activity.

During the year Dr. T. J. Phillip, of the New Hampshire Station, and Dr. J. T. Sullivan, of the Laboratory, have cooperated actively in a study of the chemical composition of certain pasture grasses. The cooperation has been wholly informal, through correspondence and conferences, but nevertheless effective in avoiding duplication of effort and keeping one another informed of progress made.

At the invitation of Dr. Fletcher, certain members of the Laboratory staff appeared before the Agricultural Experiment Station staff of the Pennsylvania State College and gave a brief verbal progress report of some of the more significant researches carried on during the year.

RESEARCH AT THE LABORATORY

Cytogenetics and Breeding

A considerable part of the work on cytogenetics and breeding has been devoted to development of technics, cytological and genetical surveys to determine what problems should be investigated, and the establishment of plant materials to be used in future studies. Although such work contributes little to fundamental knowledge, it is essential as the basis for future experimentation. Progress has been made along these lines, but the work has by no means been completed. This is particularly true in the development of cytogenetic and breeding stocks of the various species.

Attention is being focused on the permanent pasture species, <u>Poa pratensis</u> (Kentucky bluegrass) and <u>Trifolium repens</u> (small white clover); rotational pasture species, <u>Trifolium repens</u> (Ladino clover), <u>Trifolium pratense</u> (red clover), <u>Lotus corniculatus</u> (bird's foot trefoil), <u>Dactylis glomerata</u> (orchard grass), <u>Lolium perenne</u> (perennial ryegrass), and <u>Phleum</u>

pratense (timothy); and the temporary pasture species, Sorghum vulgare var. Sudanensis (Sudan grass).

Reproduction in Poa

Apomixis vs. Sexual Reproduction

As a preliminary step in studies of method of reproduction, an attempt is being made to determine the frequency of occurrence of asexual, sexual, and intermediate types in random samples of *Poa pratensis* and *P. compressa* and to isolate lines exhibiting these different types of reproductive behavior for use in further investigations. For this preliminary survey, uniformity of progenies and fidelity to the maternal plant type are used as the criteria of method of reproduction.

For the mother plants to be studied, 117 plants of Kentucky bluegrass and 23 plants of Canada bluegrass, covering a wide range of morphological types, were selected. All plants were bagged and, in addition, seed from open pollination was collected from each plant. In those plants of Kentucky bluegrass where sufficient seedlings could be obtained, 60 plants from open-pollinated seed have been established from each parent plant. In a few cases insufficient seedlings were available, but some progenies, either from self- or open-pollinated seed were obtained from all plants. No seed was produced under bag on the selected plants of Canada bluegrass, so that only progenies from open-pollinated seed were available. In the field planting, progenies of selfed and open seed and five clonal isolations of the mother plant were placed in adjacent rows to facilitate direct comparisons.

Progenies from self- and open-pollinated seed of selected plants of Kentucky bluegrass and from open-pollinated seed of selected plants of Canada bluegrass have been established as the first step in a study of the method of reproduction in these species.

Polyembryony in Poa

Polyembryony refers to the condition in which there may be two or more embryos within a single seed. This condition results upon germination of the seed in the occurrence of twin and triple seedlings. The germination of three species of Poa and the occurrence and rate of multiple seedlings are given in table 1.

Table 1.- Germination and polyembryony in Poa.

	Kentucky bluegrass	Canada bluegrass	P. alpina
Number of parent plants	117	23	4
Number of seed sown	20,189	2,400	400
Number of seed germinated	16,189	1,305	320
Percent germination, average	80.1	54.3	80.0
Percent germination, highest	100.0	95.0	97.0
Percent germination, lowest	7.2	10.0	58.0
Twin seedlings, number	1,150	76	16
Twin seedlings, percent, average	7.1	5.8	4.0
Twin seedlings, percent, highest	28.0	8.0	8.0
Twin seedlings, percent, lowest	0.0	0.0	1.0
Triple seedlings, number	39	- -	- -
Triple seedlings, percent, average	0.2	- -	- -
Triple seedlings, percent, highest	8.0	- -	- -

Of 16,189 seedlings of Kentucky bluegrass from 117 single plant progenies, twin seedlings appeared at a rate of 7.1 percent. In four plant progenies the percentage of twin seedlings was above 24 percent. In other progenies no twins appeared, indicating a cytological or genetical basis for polyembryony. Triple seedlings have appeared only in Kentucky bluegrass and there only in a few pedigrees. The average for all progenies was 0.2 percent, but in one pedigree 8 percent of triple seedlings were found. In this material the triple seedlings appeared only in those pedigrees showing a very high rate of twinning.

In Canada bluegrass the frequency of twin seedlings varied from none to 8 percent, with an average of 5.8 percent, lower than that for

Kentucky bluegrass. Here again, certain plants produced more twins than others.

Progenies from only four plants of Poa alpina were available, but among 320 seeds from these plants, 4 percent produced twin seedlings. All four plants gave twin seedlings, the highest 8 percent, the lowest 1 percent.

Twin seedlings were also found in the following species of Poa: ampla, canbyi, glaucifolia, interior, scabrella, recunda, and palustris. Percentages were low and about the same for all species, i.e., 1 to 2 percent. Species of Poa in which no twin seedlings have appeared are bulbosa, horridula, nemoralis, nevadensis, spondylodes, sterilis, trivialis, annua, and arachnifera.

The individual members of the twin and triple seedlings which were obtained are now growing as individual plants and it is apparent that, in many cases, the two members of a pair of twins, differ remarkably in size and external appearances. (See figure 1, D).

The occurrence of multiple seedlings might have a bearing on the method of reproduction in Poa. It can be seen from the data given that in the four species in which some biotypes are known or suspected of being apomictic, multiple seedlings have appeared in a high proportion of single plant progenies. These species are pratensis, compressa, palustris, and alpina. On the other hand, in annua and trivialis, which are species considered entirely sexual in method of reproduction, no multiple seedlings have appeared, yet a considerable number of seedlings of the latter species has been grown at the Laboratory.

Twin seedlings have been found in varying proportions among progenies of different plants of Kentucky and Canada bluegrass. In many cases, the two seedlings of the pair exhibited marked morphological differences.

Methodology in Controlled Pollination of Grasses

The value of hot water for mass emasculation of timothy was investigated using clonal isolations of one self-fertile plant. The heads were immersed in water varying from 44° to 54°C. and treatments were continued for 5, 10, and 15 minutes. Length of treatment did not cause observable differences. All heads were killed by temperatures of 50° C. and higher. At 48°C., some heads were killed and anthesis was abnormal in the remainder. Part of the anthers on these heads failed to dehisce. No seed was set under bag on four isolations treated at 46°C. although anthesis and dehiscence were normal. Some shriveling of the pollen was observed. Three isolations were treated with water at 44°C. and anthesis and dehiscence were normal. No seeds were set under bag on two isolations and three seeds were produced on the three treated heads of the third isolation. For control, two isolations were selfed and 40 and 48 seeds per head were produced. No pollen was available for testing viability of the ovules in treatments in which no seeds were set under bag.

Preliminary studies of a similar nature were conducted with Kentucky and Canada bluegrass. In treatments for 10 minutes at 43° and 45°C., the plants appeared to flower normally but the anthers usually failed to dehisce.

Bagging Technic

No experimental data have been collected on bagging methods with the grasses. Satisfactory results have been obtained by enclosing a number of panicles of a plant in a parchment bag (either 11½" x 4" x 2½" or 9" x 3½" x 2"). The bottom of the bag is fastened by the wire of a wood tree label and the top is secured to a cane stake by means of a string passed through a metal eyelet which has been punched in the corner of the bag.

Methodology in Controlled Pollination of Legumes

The most convenient method of bagging tried involves the use of bags of fine muslin (either $2\frac{1}{2}$" x 5" or $3\frac{1}{2}$" x $5\frac{1}{2}$") to prevent cross-pollination by bees. In the field the bags were supported by pieces of wire, 12 or 15 inches long, bent over at a little more than right angles 2 or 3 inches from one end to keep the bag stretched out at the top. In this way the top of the bag was held from 6 to 9 inches off the ground, depending on the height of the peduncles being bagged.

Large sturdy heads on which the lowermost flowers appeared ready to open within the next day or so were chosen for bagging wherever possible. In case a few flowers had started to open they were picked off. Usually two heads were enclosed in a single bag and the bag was slipped down over the wire support and the two heads and fastened firmly around the bottom. This was done by wrapping the string of a Dennison's "marking tag" around the bottom part of the bag, slipping the tag through the loop of the string and pulling the string tight. This method of fastening continued to hold when the bags were picked off for drying and threshing of the heads.

Preliminary information on the increase in seed set resulting from artificial tripping of the flowers by manipulation was obtained during the summer of 1937 (not included in the 1937 annual report because threshing was not completed). The manipulation consisted of rolling gently between the thumb and forefinger each head without removing the bag. As shown in table 2, three series of bags were put on. Those in the first series, applied between the middle of July and the first week of August, were not manipulated. The second series was put on during the last two weeks of August, and the third series during the second week of September. In the last two series, all heads were manipulated in the manner described above once each day for 5 to 7 successive days after bagging except when the bags were wet. The plants were selected for bagging more or less at

Table 2.- Self-fertility in white clover.

Series	Plants selfed	Date	Total number of Plts.	Hds.	Seeds	Ave. seeds per head	Percent of plants which set the following seeds per head: 0 Self-sterile	0-10 Pseudo-self-fertile	10-50 self-fertile	50- Highly self-fertile
I	random	7/13-8/4(1937)	76	190	90	.47	61	39	0	0
II	random	8/14-8/31(1937)	176	416	3541	8.51	22	56	19	2
III	random	9/7-9/9(1937)	88	316	851	2.69	32	59	9	0
IV	sod-plot selections	6/8-6/10(1938)	50	394	114	.29	56	44	0	0
V	other selections	6/14-6/21(1938)	165	1266	56	.04	81	19	0	0
VI	random	7/5-7/15(1938)	615	2400	13708	5.71	10	73	16	1
VII	Ladino	6/28-7/15(1938)	255	1997	459	.23	62	38	0	0

random wherever they flowered. In the unmanipulated bags an average of 0.47 seeds per head were found, while an average of 8.51 seeds per head were obtained in the manipulated bags of series II, the amount of selfed seed having been increased over 18 times by manipulation. The decrease to 2.69 seeds per head in series III is thought to be principally the effect of the later season.

Further information on methodology in selfing was secured this past summer. On the basis of the selfed seed obtained in the field during the summer of 1937 and in the greenhouse during the winter of 1937-38, seven plants were chosen in each of the following four classes:

(1) Practically self-sterile (0-1 seeds per head)

(2) Pseudo-self-fertile (1-10 " " ")

(3) Self-fertile (10-50 " " ")

(4) Highly self-fertile (50+ " " ")

Four bags, each enclosing two heads, were tied on each of the 28 plants on the same day. One bag on each plant was manipulated six times (every

day for six days after bagging), the second three times (every other day for six days), the third one time, and the fourth not at all.

At all three dates one manipulation increased the average seed set per head over no manipulation (see table 3.) Unexpectedly six manipulations gave considerably fewer seeds per head than the no manipulation at every date. At the first date the three manipulations gave the highest seed set, while at the other two dates the one manipulation proves somewhat the best.

Table 3.- 1938 manipulation study.

Date of bagging	Number of manipulations	Average seed set per head - percent: Self-sterile	Pseudo-self-fertile	Self-fertile	highly self-fertile	Average of the four classes
6/7/38	0	.07	.07	.64	16.75	3.93
	1	1.07	.15	5.43	20.84	6.84
	3	.14	.29	9.69	40.55	11.12
	6	.00	.08	1.23	4.46	1.42
Average		.32	.15	4.20	19.91	5.77
6/22/38	0	.27	.17	1.89	1.90	.98
	1	.00	.17	2.55	7.56	2.33
	3	.00	.00	.70	3.11	.85
	6	.00	.00	.56	.00	.12
Average		.07	.09	1.46	3.11	1.08
7/20/38	0	.82	3.90	1.60	6.78	3.12
	1	.50	.92	3.18	13.00	3.84
	3	.00	1.11	1.00	2.57	1.03
	6	.00	.00	.00	.00	.00
Average		.33	1.46	1.45	5.97	2.06
Average		.25	.51	2.56	10.82	3.23

At these latter two dates three manipulations were inferior to no manipulation. A seasonal effect was also noted in that the pseudo-self-fertile plants had a higher seed set at the last bagging than at the first two, but the self-fertile and highly self-fertile plants produced more seeds per head at the first bqgging.

Manipulation in Lotus corniculatus was accomplished in a different manner. Wire supports were used to hold the bag off the ground and the bags were tied over the inflorescences before the flowers opened, but pollination could not be done effectively by simply rubbing the head. When all flowers on each inflorescence had opened on the fourth day after bagging, the bags were removed long enough to trip each flower by pressing a small pointed piece of filing card against the side of the keel facing the standard. At no time does the keel spring open exposing the inner floral parts.

Fifteen second-year plants of bird's foot trefoil were bagged. Eight bags, wach covering one inflorescence, were put on each of the plants. The flowers in four bags were manipulated as described above, while the other four were not manipulated. No seeds were found in the unmanipulated bags, but a total of 208 seeds were harvested from the manipulated series.

Controlled self-pollinations with the clovers in the greenhouse were made by rolling the heads the same as in the field except that no bags were used during the winter when bees were not flying in. Precautions were taken to prevent contamination by wiping the hands with alcohol between rubbing different plants.

A more elaborate technique has been worked out for controlled crosses, all of which have been made in the greenhouse. Flower heads are selected for crossing when about half of the flowers have opened. All but ten of the most recently opened flowers are pulled off. These are emasculated by the suction force from a vacuum pump obtained through a

capillary glass tube drawn out to an opening of $\frac{1}{4}$ - $\frac{1}{2}$ mm. When emasculating, binocular loupe magnifiers are worn to facilitate operations. The keel of a flower is pulled gently away from the stamenal column and held down by the thumb and forefinger after which the end of the capillary is brought close to the anthers which are sucked off without being ruptured. In case an occasional anther is broken as the petals are pulled back, all the loose pollen, which usually sticks together in fairly large clumps in recently opened flowers, can be easily cleaned off with the capillary. Under no circumstances is it necessary to touch the stigma or style with the capillary. Pollen is collected from another plant by tripping one of its flowers onto a small piece of very fine grade of emery paper which has been glued onto the broad end of a toothpick. For pollination of the emasculated flowers the keel is again held away from the pistil and the emery paper rubbed gently on the stigma.

Last winter 66 crosses involving 70 parent plants were made in this manner. Seed was obtained in all except one and in this case both plants came from the same seed lot, which may mean that they were related in some degree. The seed set ranged from seven (excluding the one sterile cross) to 55 with a mean of 34.6 seeds per cross or approximately $3\frac{1}{2}$ seeds per pod. This number of seeds is much higher than that obtained with open-pollination of entire heads in the field or with self-pollination of entire heads of even the most self-fertile plants. As a check on the emasculating technique, 14 heads on as many different plants were emasculated but not pollinated. Not a single seed was obtained.

Parchment bags have been satisfactory for obtaining selfed seed of the grasses. In the legumes, cloth bags are used but artificial pollination is essential for good seed set. In white clover this is accomplished by rolling the head gently to trip the flowers. Hot water for emasculation of the grasses and suction for emasculating white clover are being investigated.

Self Pollination in Grasses

A survey was made of the ability of plants of *Lolium perenne*, *Dactylis glomerata*, and *Phleum pratense* to set seed under bag. As can be seen in table 4, a wide range was exhibited by plants of each species. Of the

Table 4.- Range in ability of plants of *Lolium perenne*, *Dactylis glomerata* and *Phleum pratense* to set seed under bag.

Species	Number of seed per head												Total
	0	1-	1-5	6-10	11-15	16-20	21-30	31-50	51-100	101-150	151-200	Over 200	
L. perenne													
Number of plants	119	266	438	110	33	11	10	2	-	-	-	-	989
Percent of plants	12.0	26.9	44.3	11.1	3.3	1.1	1.0	0.2	-	-	-	-	
D. glomerata													
Number of plants	39	51	142	69	46	22	33	40	38	12	3	2	497
Percent of plants	7.8	10.3	28.6	13.9	9.2	4.4	6.6	8.0	7.6	2.4	0.6	0.4	
P. pratense													
Number of plants	122	54	114	27	12	14	6	12	10	4	4	4	383
Percent of plants	31.8	14.1	29.8	7.0	3.1	3.6	1.6	3.1	2.6	1.0	1.0	1.0	

989 plants of perennial ryegrass, 119 plants, or 12.0 percent, failed to set seed. An additional 26.9 percent averaged less than one seed per head. On the other extreme, two plants produced 33 and 42 seeds per spike. Orchard grass showed considerably less self-sterility with 7.8 percent of the plants failing to set seed and an additional 10.3 percent averaging less than one seed per head. Two plants of the 497 that were tested set more than 200 seeds per head. In timothy, out of 383 plants that were bagged, 122, or 31.8 percent, did not set seed and 14.1 percent set less than one seed per head. On the other hand, four plants averaged more than 200 seeds per head. One of these plants produced 658 seeds on a single head. It is evident from these results that plants showing a considerable degree of self-fertility occur in all these species.

During the fall of 1937 plants of Kentucky and Canada bluegrass were brought into the greenhouse. All were first-year plants; no plants of Kentucky gluegrass flowered in the field, but some of the Canada blue-

grass plants had flowered during July and August. Each of these plants was planted in a three-foot row in a greenhouse bed.

All plants that flowered were bagged with parchment bags. The seed set under bag was classified as (1) none, (2) poor, (3) fair, and (4) good. The results are given in table 5. Canada bluegrass was highly sterile when bagged. About 56 percent of the plants set no seed and only about 7 percent were classified as good. On the other hand, Kentucky bluegrass seet seed well under bag. Over 60 percent of the plants were classed as good and only about 20 percent failed to set seed.

Table 5.- Seed set in Poa pratensis and P. compressa under bag.

	Number of plants bagged	Seed set: None	Poor	Fair	Good
Kentucky bluegrass:					
In greenhouse, 1937-38	61	12	8	4	37
Percent of plants		19.7	13.1	6.5	60.6
In field, 1938	538	66	63	58	351
Percent of plants		12.3	11.7	10.8	65.2
Canada bluegrass:					
In greenhouse, 1937-38	70	39	15	11	5
Percent of plants		55.7	21.4	15.7	7.1
In field, 1938	218	104	76	17	21
Percent of plants		47.7	34.9	7.8	9.6

During the spring of 1938, 218 plants of Canada bluegrass and 538 plants of Kentucky bluegrass were bagged in the field. The plants of Canada and Kentucky bluegrass used in the greenhouse during the winter were included. The data obtained from these plants are summarized in table 5. It can be seen that the results from greenhouse and field studies are in close agreement. Under field conditions about 48 percent of the plants of Canada bluegrass set seed and about 10 percent were classed as good. In Kentucky bluegrass, on the other hand, about 12

percent of the plants failed to set seed under bag and over 65 percent were quite fertile.

Sudan grass selections were secured from South Dakota, Wisconsin, Colorado, Texas, and the United States Department of Agriculture plots at Beltsville, Maryland. Open-pollinated, commercial seed was obtained from Texas, Illinois, and Nebraska.

From 500 to 600 plants were self pollinated in the field during the summer of 1938. From these selfs there are now available 250 lines which have been inbred two or more years and 150 lines inbred one year.

For studies of the inheritance of self-fertility and sterility, and the effects of inbreeding and as a first step in the establishment of inbred lines, 60 families of the first inbred generation have been established in orchard grass and in perennial ryegrass, and 79 families have been planted in timothy. These families are progenies of plants which ranged from nearly completely self sterile to as highly self fertile as were obtained. Thirty plants, planted in 3 rows which were distributed at random in 3 replications, were grown from selfed seed of each parent. In the rows adjacent to the inbred progenies, clonal isolations of the mother plants were planted to facilitate accurate determinations of the effects of inbreeding and inheritance of self fertility.

From each of 26 of the most self-fertile plants of orchard grass, included in the experiment described above, 70 additional progenies are being grown from seed produced under bag. In addition, 100 plants from selfed seeds produced on 43 other plants of orchard grass and 30 progenies from each of 68 other plants of perennial ryegrass were planted. The purpose of these plantings was to give additional inbred progenies from to select for the production of inbred lines and from which mutant genes could be obtained.

Varying degrees of self fertility have been found in different plants of the grass species which have been studied. Progenies of the first inbred generation have been established in several species.

Self Pollination in Legumes

The data on seed set under bag of plants of white clover are summarized in table 2. Series I, II, and III were plants bagged in 1937 as already explained in an earlier section of this report. These plants were selected as representatives of the range of morphological variation. The plants in seried I were not manipulated and the results are therefore not indicative of their potentialities. Series IV, V, VI, and VII were bagged in 1938. Plants in seried IV and V were selected to represent the range of growth types found in the field while those in series VI were taken at random by selecting the first group of plants that flowered within each seed lot. The Ladino plants were selected as representatives of the range of morphological types.

The highest fertility was obtained in series II in 1937 and series VI in 1938. In series II, less than one-fourth of the plants were highly self sterile and a similar number set more than 10 seeds per head. The remainder were classed as pseudo-self-fertile. In series VI, only 10 percent of the plants failed to set seed, but the percentage of plants classed as self-fertile was lower than in seried II. The remaining series were much lower in seed set than series II or VI. However, it appears that these differences may have been conditioned by environmental or manipulation factors or both rather than by inherent differences in self-fertility. Plants in series IV, V, and VII were manipulated six times by the same worker who was responsible for the manipulation study (see table 3) in which six manipulations were inferior to no manipulations. Series VI was manipulated by another worker.

That self-fertility is a definite character which can be worked with, even though it is markedly influenced by the environment, is best shown by the data in table 3. Plants were selected for the four fertility classes on the basis of their 1937 field performance and their seed set in the greenhouse last winter. The plants fall again into the same four relative classes, although the actual 1938 seed set was somewhat less. Despite the variability in this character of self-fertility, it should be more useful in breeding studies than might be predicted from William's results at Aberystwyth. When his flowers were unmanipulated, 88 percent failed to set seed as contrasted with 61 percent in series I. Even when he artificially pollinated each flower separately (not simply rolling the entire heads as here), 74 percent of his population was self-sterile as against 10 percent in series VI or 22 percent in series II. Apparently some inbred seed can be relatively easily obtained from a large proportion of any population.

Only 15 plants of bird's foot trefoil were bagged last summer to test their self-fertility. They were all in their second summer's growth. From the four inflorescences bagged on each plant, three plants set no seed, four set less than five seeds, four set from six to 25 seeds, and four set more than 25 seeds.

Plants of the first inbred generation were set out in the 1938 nursery in preliminary inbreeding experiments. Twenty-six plants set enough seed in 1937 to produce 30 progeny from each. The inbreds were planted in three randomized blocks in the manner described previously for the grasses. This will be referred to as inbreeding experiment No. 1. Also 12 selected plants grown in the greenhouse last winter produced at least 30 progeny each. These were planted the same as experiment No. 1. This will be called inbreeding experiment No. 2. In addition, 30 plants of each of four lines of second generation inbreds were planted as inbreeding experiment No. 3 in the same way as the first two groups. These

two-year inbreds came from four sister one-year inbreds which in turn came from a single plant that flowered in the greenhouse during the winter of 1936-37. Finally in the field there were planted several inbred lots of less than 30 plants which came from pseudo-self-fertile parents.

Notes were taken during August on the inbredlots in all three sets of randomized blocks. The plants were scored for general vigor as shown principally by the amount of ground covered and the number of leaves produced. The average vigor of each row of five parent clones was arbitrarily classed as ten. The adjacent progeny were then rated on comparative basis with marks of one to ten, one being the smallest survivors in field, ten being equivalent to the parents. As shown in table 6, the mean rating of

Table 6.- Vigor rating of inbreds.

Inbreeding experiment	Total number of inbreds	Mean rating of inbreds compared with parents as 10	
No. 1	690	7.11	
No. 2	228	6.68	7.00
No. 3	102	6.08	

the 918 inbreds in experiments No. 1 and No. 2 was 7.00 as compared with 10 for each of the respective parents. In other words, this group of 38 first generation inbred lines showed an average decrease in vigor of 30 percent. In table 7 the frequency distribution of these 38 lines is shown.

Table 7.- Frequency distribution of 38 first-generation inbreds rates on vigor in comparison with parents.

3	4	5	6	7	8	9	10	Total
1	3	6	11	5	7	4	1	38

One line was practically as vigorous as its parent, while another lost 70 percent in the first generation. Since the four second-generation inbred

lines in experiment No. 3 are closely related, the 40 percent decrease from the first to second generation shown by these plants (see table 6) may not hold for a larger population.

In connection with the inbreeding program, it must be decided whether an attempt should be made to obtain selfed seed during the first summer's growth of the inbreds, or whether the selection of plants to be inbred further should be postponed at least until the second year. An indication as to what the first alternative will eventually lead to may be obtained from the data on flowering in table 8. It is clearly evident that the inbreds

Table 8.- Flowering data - August 1938.

Experiment		Total number of plants	Percent of plants which flowered as follows: None	Sparse	Medium	Profuse
Inbred progenies	No. 1	694	13	10	28	50
	No. 2	227	47	22	17	14
Parent clones	No. 1	26 (15 clones each)	0	8	15	77
	No. 2	12 (15 clones each)	0	33	50	16
Sod-plug isolations		804	46	18	22	13

in experiment No. 1 flowered much more profusely than those in experiment No. 2. Those in experiment No. 1 came from plants which had flowered in their first summer. Those in experiment No. 2 came from plants which did not bloom profusely enough in their first summer (some did not flower at all) to give the required 30 seeds, but did flower during the following winter in the greenhouse. No count was made during the summer of 1937 of the total number of plants which flowered in the entire population of

10,000. It was noted, however, that of the 250 plants selected as representing the range of morphological variation 35 percent failed to flower during that first summer. By inbreeding those that did flower, apparently plants were selected whose offspring flowered more profusely in their first summer than offspring derived from the greenhouse flowering plants. In the same way the parent clones in experiment No. 1 flowered more profusely than those in No. 2. The best check on this clonal material was in the sod-plug isolations, which were started as slips at about the same time as the parent clones. The sod-plug isolations must have been at least one year old, the same as the parents of the inbreeding experiment, and it is quite probable that they were much older. If age alone is the conditioning factor, the material from sod-plugs should have bloomed more than the parent clones in their second year, but actually 46 percent of the sod-plug isolations failed to flower in 1938, whereas none of the parent clones failed to flower.

Plants of white clover varied from completely self-sterile to highly self-fertile. Environmental factors and manipulation seemed to greatly influence the amount of seed set from self pollination. First-generation inbred progenies averaged 30 percent reduction in vigor as compared with their parents.

Hybridization of Grasses

Intergeneric Hybrids

In several attempts to cross Lolium perenne with Festuca elatior, seed set was obtained in most crosses, particularly when F. elatior was used as the female parent. In all but one case the seed failed to germinate. In this cross, no seed was set when F. elatior was used as the female but 12 seeds were obtained with L. perenne as the female parent. These seeds were delayed in germination and exhibited considerable variability in this respect. Nine seeds germinated and produced vigorous seedlings,

all of which lived and appear morphologically to be hybrids.

Intraspecific Hybrids in Lolium perenne

Intercrosses of self-sterile plants of Lolium perenne are being studied as one approach to the determination of the cytogenetic basis of self and cross incompatibility. In a preliminary study, 26 crosses and reciprocals were made involving 23 different plants. In two crosses, no seeds were set in either reciprocal. The remaining crosses set seed in one or both directions.

Later, 19 self-sterile plants were selected and an attempt was made to cross these in all combinations. Of the 171 possible crosses, 165 were made. Of these 12 failed to produce seed in either reciprocal, in 50 seed set was high in one reciprocal and low in the other, 22 set seed in one direction but not in the other, in 59 seed set was low in both directions, and in 22 high seed set was obtained in both reciprocals. No genetic explanation of the results is available at present.

Hybrids of Lolium perenne and Festuca elatior have been obtained. Intercrosses of self-sterile perennial ryegrass plants varied from failure to set seed to high seed set.

Hybridization of Legumes

Interspecific Crosses

Attempts were made to cross white clover with red, alsike, and zizag clover, but no hybrid seedlings were obtained.

Intraspecific Crosses in White Clover

Under "Methodology in Controlled Pollinations" the number of crosses made in the greenhouse last winter and results obtained are tabulated. All of the crosses were between unrelated plants and all were fertile (one ex-

ception and the possible cause was noted).

Last winter ten flowers on one head of each of 13 white clovers were crossed with different Ladino plants. Altogether 512 seeds were obtained. The average of over 39 seeds per cross was higher than that from intraspecific white clover crosses. The reciprocals also set seed but not so well. Apparently this intervarietal cross can be easily made.

Interspecific hybrids in the genus Trifolium have not been obtained. Intraspecific hybrids in white clover were fertile in all except one case. White clover crossed readily with Ladino clover.

Preliminary Genetic Studies in Grasses

In the grasses, particular effort in isolating qualitative characters of value as chromosome markers has been confined to Lolium perenne and Dactylis glomerata. A number of such characters have been found but their inheritance has not yet been determined.

Among inbred progenies of 125 plants of perennial ryegrass which were studies, 21 were segregating for white seedlings, four contained yellow seedlings, one had seedlings which were white except for green-tipped leaves, and one had light green seedlings. In addition, one family segregated in the ratio of 32 green:6 yellow:1 white. In the progenies segregating for white seedlings, the ratios varied from 11 green: 16 to 56 green:1 white, but in most cases the ratios approached the theoretical 15:1 or 3:1. The results indicate that different factors are conditioning the white seedling character and perhaps that different types of factor interaction are involved. The ratios obtained for the other characters, yellow, green tips, and light green, approach a 3:1 ratio with deficiency of the recessive class.

Some additional seedling characters which were observed but for which definite ratios were not obtained were red vs. green base, wine

colored leaf blades particularly near the base, variegated leaves, and leaves rolled in the bud instead of folded. In older plants, some characters which may prove useful are colored leaf sheaths, colored nodes, colored vs. green anthers, annual vs. perennial habit, erect vs. decumbent growth type, presence and absence of awns, and rust resistance vs. rust susceptibility.

Seedlings of inbred progenies of 78 orchard grass plants were examined and 20 were found to contain white seedlings. The ratios varied from 122 green:24 white to 151 green:1 white. Only two other progenies showed ratios of less than 25:1. The great predominance of green seedlings in most segregating families may be significant in relation to the cytological behavior of this species. As stated later in this report, orchard grass behaves cytologically as an autotetraploid. Tetrasomic instead of disomic ratios would be expected and the results obtained may be explained on that basis. Several characters have been obtained for which inheritance data are not available. Plants were found which appear light green, particularly during the cool weather of the early spring. Another more yellowish green plant was obtained from a small inbred family and plants with grayish-green leaves were found in another inbred family. All of these plants have good vigor. Additional characters are color of glumes, pubescence of the keel of the lemma, color of anthers, color of nodes, variegated leaves, and rust resistance vs. rust susceptibility.

Seven male sterile plants of Dactylis glomerata have been found and F_1 hybrids of one of these with a normal plant are now available for study.

A number of differential characters have been found in perennial ryegrass and orchard grass, but their inheritance has not been determined. Preliminary evidence suggests tetrasomic ratios in orchard grass.

Preliminary Genetic Studies in Legumes

In addition to the studies on self and cross compatibilities, several other genetic problems are just getting under way. In white clover, several morphological and physiological characters which may be useful in linkage studies or as markers are being investigated. At present the characters which appear most promising are (1) unifoliolate leaf, (2) red midrib, (3) white markings on leaflet, (4) pattern of markings on leaflet, (5) solid white inside of markings on leaflet, (6) red specks on leaflet, (7) diffused purple color on leaflet, (8) red flower color (several shades), (9) purple seed color, (10) fasciated stolons, (11) length of internode, (12) chlorophyll deficiency at low temperature, (13) presence of HCN, and (14) proportion of floral to vegetative buds. Inbreeding is being attempted by both selfing and sibbing with plants carrying these characters. When homozygous lines have been established, crosses will be made and inheritance of the characters settled.

Last summer in the field the unifoliolate plant flowered for the first time. From over 25 heads bagged for selfing only 1 seed was obtained. A number of open-pollinated heads bearing an abundance of seed were gathered, and seed was set in several controlled crosses made in the greenhouse between normal trifoliolate plants and the unifoliolate plant, using the latter only as the pollen plant.

Preliminary data indicate that the presence of HCN is a definitely heritable character. (See Physiology and Composition.) First generation inbreds have been found completely free of HCN when the parent also gave a negative test. Two second-generation inbred lines have given positive tests throughout and their parents were positive. In this latter case there was some difference between sisters in the amount present, which might indicate that the presence of the glucoside is determined by several genes. More information supporting this conclusion was that all

of the approximately 70 plants tested from seed lot No. 62 gave a positive test, but in varying degrees.

A correlation was observed last summer between the paucity of leaves on some plants and the number of flower heads they had produced. Notes were taken on 15 plants selected for predominance of floral branches and on 8 plants selected for predominance of vegetative branches. Two of the latter plants were outstanding in the field of 10,000 in their production of practically 100 percent vegetative branches, while almost every other plant in the field flowered profusely during the summer. The amount of apparently continuous variation in this character might indicate that it is determined by several genes. Two heads have been crossed between a predominantly floral and a predominantly vegetative plant and more will be crossed when flowers are available.

Several differential characters have been found in white clover and work is now in progress to obtain these in the homozygous condition for use in genetic studies. Some evidence has been obtained which indicates that HCN content is heritable.

Reduplication of Chromosomes in Grasses

Seeds of Lolium perenne were germinated for 6, 24, and 96 hours in Petri dishes on blotting paper moistened with 0.1, 0.2, and 0.4 percent aqueous solutions of colchicine. At the end of the treatment, the seeds were transferred to blotters wet with tap water for completion of germination, after which the seedlings were transplanted to soil. After the plants had grown to maturity, four tillers were broken from each and the new root tips produced by these clonal isolations were examined cytologically.

As shown in table 9, the colchicine treatments delayed germination, reduced the total number of seedlings obtained from the 60 treated seeds, and resulted in the production of abnormal seedlings. These effects were

Table 9.- Number of root tips visible at the end of 48, 60, 72, and 84 hours, number of normal and abnormal seedlings, and total number of seedlings obtained from 60 seeds with different concentrations of colchicine and lengths of treatment.

Treatment	Percent	Hours	No. of root tips visible at end of indicated hours: 48	60	72	84	Total number of seedlings obtained: Normal	Abnormal	Total
Colchicine	0.1	6	22	37	53	56	52	8	60
	0.1	24	8	22	41	46	36	22	58
	0.1	96	3	7	14	21	0	30	30
	0.2	6	20	34	53	55	50	10	60
	0.2	24	4	11	33	38	23	36	59
	0.2	96	0	0	3	9	0	34	34
	0.4	6	10	21	45	51	44	15	59
	0.4	24	0	6	26	36	17	38	55
	0.4	96	0	0	0	2	0	20	20
Tap water	- -	- -	28	39	52	54	55	4	59

accentuated with the higher concentrations of colchicine and with longer durations of treatment.

The incidence of tetraploid sectors among plants from the different treatments is given in table 10. In transplanting the seedlings, 20 were

Table 10.- Incidence of plants producing 4n root tips, from random and selected seedlings, following treatments or germinating seeds with colchicine.

Colchicine solution	Number of plants	Duration of treatment: 6 hours R 1/	6 hours S 2/	24 hours R	24 hours S	96 hours R	96 hours S	Total R	Total S
Percent									
0.1	Examined	20	2	17	3	1	0	38	5
	4n sectors	0	0	2	0	0	0	2	0
0.2	Examined	18	2	16	13	1	1	35	16
	4n sectors	0	0	6	6	1	0	7	6
0.4	Examined	18	6	13	15	8	4	39	25
	4n sectors	1	1	5	6	5	2	11	9
Total	Examined	56	10	46	31	10	5	112	46
	4n sectors	1	1	13	12	6	2	20	15

1/ Seedlings selected at random

2/ Seedlings selected because of abnormal appearance.

taken at random from each treatment and then all remaining abnormal seedlings were selected. The data from plants from these two classes are recorded separately. Treatment for 6 hours was relatively ineffective in producing 4n sectors. Likewise, the 0.1 percent solution produced only two plants out of 43 with tetraploid tissue. Treatments for 24 hours with the 0.2 and 0.4 percent solutions of colchicine were the most satisfactory from the standpoint of seedling survival and incidence of induced polyploidy.

The appearance of the primary root (see figure 1, B) and of the coleoptile and plumule of the seedlings was classified as normal or abnormal. Later, the appearance of the mature plants was recorded in the same manner. The relation of incidence of tetraploidy to the appearance of the seedlings and mature plants is shown in table 11. In general the plants showing 4n sectors were abnormal in growth type as seedlings and mature plants. These abnormalities were not reliable criteria of chromosome doubling since many abnormal appearing seedlings and plants did not produce 4n roots or flowers.

Table 11.- Relation of incidence of tetraploid sectors to abnormal appearance of the primary root and coleoptile and plumule of the seedlings, and to the abnormal appearance of the mature plant.

Appearance of	Number of plants		Total plants
	Completely 2n	Containing 4n sectors	
Primary root-			
Normal	71	2	73
Abnormal	52	33	85
Coleoptile and plumule-			
Normal	50	1	51
Abnormal	73	34	107
Mature plant-			
Normal	99	6	105
Abnormal	24	29	53
Total	123	35	158

The proportion of diploid and tetraploid tissue in the different plants can be determined approximately from the number of clonal isolations of each which produced 4n root tips. These data are summarized in table 12.

Table 12.- The proportion of tetraploid and diploid tissue in plants in which chromosome duplication was induced, as indicated by the number of clonal isolations from each plant that produced 4n root tips.

	Number of plants producing indicated number of 4n isolations				
	1	2	3	4	Total
Number of plants	7	8	8	9	32

In addition to the 32 plants recorded in this table, from each of three plants three isolations produced 2n root tips and the fourth produced one 2n and one 4n root tip.

Plants which produced 4n root tips also produced large pollen grains. Tetraploid progenies were obtained from seed produced on plants having 4n root tips and large pollen grains. In addition, one triploid plant was obtained, probably as a result of pollination of a tetraploid flower with pollen from a diploid plant.

From those plants which were chimeras of 2n and 4n tissue, root tips from single culm isolations have been examined. Diploid and tetraploid clones from the same plants are now being grown for cytogenetical, morphological, chemical, and physiological studies. Since the 2n and 4n clones from each plant have the same gene base, any differences found in comparative studies should be the result of chromosomal or gene duplication. The data obtained to date indicate that the 4n sectors have more robust but fewer culms, and longer and broader leaves.

Seeds of diploid Festuca elatior and diploid Agropyron cristatum have been treated with colchicine and abnormal appearing seedlings were obtained. Cytological examination of plants from these treatments has

not yet been made.

The application of colchicine by injection with a hypodermic needle into the center of the culms has been investigated on a limited scale. Of three plants of Lolium perenne treated in this manner, one showed a 4n sector. More extensive studies of this method are now in progress.

In studies of the effects of heat treatment of germinating seeds on inducing chromosome doubling, seeds were held at 7°C. until cell divisions started and then were transferred immediately to 40° to 44°C. From these treatments, 67 plants of Lolium perenne and 42 plants of Festuca elatior have been examined cytologically. No tetraploid sectors were found.

Tetraploids have been obtained by treatment of seeds of Lolium perenne with solutions of colchicine and by injection of the colchicine solutions into the tillers of young plants.

Last winter a few seeds of Sudan grass were treated with 0.2 and 0.4 percent solutions of colchicine for 24 hours. Sixty-nine of the resulting plants flowered and set selfed seed. At least one seedling was obtained from 52 of these progenies, and root tips from them were examined cytologically. All were counted as normal 20 chromosomes except two which may have 21. These last two plants are still alive and further study will be made to check the accuracy of this observation. Some interesting notes on chromosome morphology and nucleolar behavior were made in this study, which might suggest that Sudan grass deserves further work in this respect.

Reduplication of Chromosomes in Legumes

In an attempt to double chromosome number, treatments of red and white clover were made last winter using 0.1, 0.2, and 0.4 percent solutions of colchicine in tap water. Both species were treated in the following two ways: (1) Scarified seeds were treated in the same manner as described for Lolium perenne; and, (2) drops of the three concentrations of colchicine

solution were placed on the stem apex of normal seedlings growing in soil between the time when the cotyledons were completely opened out and the time when the first trifoliolate leaf appeared. Since the stipules on the unifoliolate leaf are very inconspicuous, the meristem is unprotected during this period from the colchicine drops balanced on the top. Three durations of treatment were used, consisting of two, four, or eight drops applied at hourly intervals. Twenty seeds or seedlings were used in each of the 18 types of treatment.

In red clover, the seed treatment produced many abnormal seedlings with small, dark-green, thick, rough leaves and with very swollen root ends. This latter abnormality appeared to inhibit growth to such an extent that from 25 to 100 percent of the plants died in the different treatments. In addition, as the plants grew older, normal-appearing tissue seemed to outgrow the abnormal, so that only two mature plants were obtained which are completely tetraploid. One was obtained in the 6-hour treatment with 0.4 percent and the other in the 96-hour treatment with 0.1 percent. Both of these plants have very wrinkled leaves (see figure 1, C) and have grown very slowly. One of them flowered last summer in the greenhouse; it was self-pollinated and crossed with both normal 2n red clover and white clover, but no seed was set. No polyploids were found among the drop-treated seedlings.

In the white clover, on the other hand, no polyploids were found in the seed-treated material. Abnormal leaves were observed on the seedlings, but here there was even a greater tendency than in the red clover for the normal-appearing tissue to supplant the abnormal parts. All of the plants eventually appeared quite normal, and no polyploids were found. Consequently, when the plants treated with drops showed the same mixture of normal and abnormal parts, the normal-appearing stolons on twenty plants promising plants were cut off, thus forcing the abnormal stolons or axillary

buds on them to develop. At least seventeen of these twenty plants have given cytological evidence of polyploidy by chromosome counts in root tips, by the production of irregular and larger pollen, or by increased stomatal size. All of the nine treatments were represented in the 20 plants except the treatment with 2 drops of 0.1 percent, which treatment produced no promising plants. Among the seventeen with evidence of polyploidy, at least one traces back to each of the eight treatments included among the selected plants. In July, ten single node or terminal bud slips were taken from ten different stolons of each selected plant. Chromosome counts are now being made on root tips taken from these slips. Several irregularities, in addition to the doubling from $2n = 32$ to $2n = 64$, have been found. Of eight slips counted from one plant, one had 32 chromosomes, six had about 64, one had about 128, and some seemed to have 70 or 72 chromosomes. Another interesting feature found is the fact that different 64-chromosome slips from different parts of the same original plant grow quite differently. Some grow practically as vigorously as sister 32-chromosome slips, while others have produced only about one leaf per month since they were started as slips. Measurements of stomatal size have been made on all of the slips, but the chromosome counts are not sufficiently complete yet to warrant final conclusions. The observations so far indicate that increased stomatal size does not always accompany the doubling of chromosomes. For example, one 32-chromosome slip had larger stomata than a 64-chromosome slip from the same original plant. In general, however, a positive correlation between larger stomata and chromosome doubling was observed. Most of the twenty selected plants flowered sparsely in the greenhouse last summer, and open-pollinated seed was obtained from most of those that flowered. Some of these seeds have been germinated, and root tips from the resulting plants are now being prepared for cytological study.

Tetraploid plants of red clover and octoploid plants of white clover have resulted from colchicine treatment. In addition, other effects appear to have been obtained in white clover.

Other Chromosome Studies in Grasses

Cytology of Natural Autotetraploids

Although polyploidy is of common occurrence in most plant families, relatively few species behave cytologically as autopolyploids. In contrast with this, three species of pasture grasses, namely, Dactylis glomerata, Agropyron cristatum, and Arrhenatherum elatius, show this type of behavior. All three are tetraploid with 28 chromosomes.

Studies of meiotic behavior show striking similarities in these species as regards formation of quadrivalents. The number of gradrivalents found at metaphase I of meiosis varies from one to seven for different microsporocytes of the same plants in each of the three species. The average number of quadrivalents per sporocyte varies slightly for different plants but is about 3.5 for each species. The formation of quadrivalents and occasional trivalents and univalents in meiosis leads to irregular chromosomal distribution, to the production of aneuploid gametes, and, finally, to the production of aneuploid plants. Chromosome numbers have been determined to date for 36 plants of Dactylis glomerata. One plant had 27, 23 had 28, 8 had 29, 3 had 30, and one had 31 chromosomes. Thus, 13 out of 36 plants had more or less than the normal number of chromosomes.

Chromosome Morphology in Lolium perenne

Studies of chromosome morphology in prophase of the first microspore mitosis indicate that the seven chromosomes of the haploid set Lolium perenne may be distinguishable microscopically. Preliminary measurements have shown

that the ratio of length of the longest to the shortest is approximately 2:1. The ratio of arm lengths of the different chromosomes varies from about 1:1 to about 2:1. The work of McClintock with maize and Heitz and others with a number of other species has focused attention on the role of a particular body, the nucleolus organizer, in nucleolus formation. In most haploid chromosome sets, only one member bears a nucleolus organizer which is frequently near the end, thus accounting indirectly for the occurrence of satellites. In Lolium perenne two chromosomes of the haploid set bear nucleolus organizers. In one case the organizer is nearly median and in the other it is sub-median. In resting nuclei of diploid L. perenne, four nucleoli are possible and occasionally occur. Usually various degrees of nucleolar fusions occur so that one, two, or three nucleoli are found. In tetraploid plants of L. perenne eight nucleolus organizers are present in somatic cells but eight nucleoli have never been observed in root tip cells probably because of nucleolar fusions.

Some attempts have been made to study midprophase of meiosis in Lolium perenne. Difficulties are encountered as a result of the small cell size as compared with the amount of chromatin material. As a result, figures in which most of the chromosomes can be traced are rare. Nevertheless, the results indicate that midprophase studies may be useful in some cases in this material. No prominent pycnotic knobs were found and the spindle fiber attachment regions were not as conspicuous as in maize. Characteristic differences in chromomere size were observed.

Cytological studies of naturally occurring autotetraploids and of chromosome morphology in Lolium perenne are being made.

Other Chromosome Studies in Legumes

Chromosome counts are being made on most of the plants used for other studies, especially those plants showing various phenotype variations.

Since white clover is a naturally occurring tetraploid, plants with aneuploid numbers might be present as a result of irregular chromosome assortment. None have been found to date. Studies of chromosome morphology have proven difficult in white clover because of the small size and large number of chromosomes. Preliminary work with diploid legume species indicates that they are better suited for this purpose.

Smears of pollen mother cells in white clover have not been very successful because of the difficulties in dissecting flowers. Apparently a few quadrivalents are formed, but the details have not been ascertained. Some permanent slides were made last winter, but in the slides prepared so far no fixative has given outstanding results. Preliminary attempts have also been made to develop a rapid technique for staining the stylar tissue with pollen tubes intact. In this way a larger number of styles could be examined for relative measurements of pollen tube growth than if the more laborious process of permanent slides had to be used.

Chromosome numbers and meiotic behavior have been investigated in white clover.

Evaluation of Plant Types

Sod Plots

As a preliminary step in evaluating individual plants from the standpoint of their adaptibility for pastures, plots have been established from clonal increases of different plants of *Poa pratensis*, *P. compressa*, *Trifolium repens*, *Phleum pratense*, *Dactylis glomerata*, and *Agrostis* species. The clonal increases were started in flats in the greenhouse in the spring and as soon as they were well established, were transplanted to the field. Plots 3' x 7' were used and the cuttings were placed 7 inches apart each way for the grasses and 12 inches apart each way for the clover. Plots were established for 81 plants of Kentucky bluegrass, 64 of Canada blue-

grass, 64 of white clover, 36 of timothy, 36 or orchard grass, and 36 of red top. The plots are in quadruplicate for Kentucky and Canada bluegrass and white clover, and in duplicate for timothy and red top. Only a single plot was planted from each plant of orchard grass. A uniform seeding of small white clover was made with Kentucky and Canada bluegrass, while Ladino clover was seeded with timothy and orchard grass. Kentucky bluegrass was seeded with the white clover plots.

All plots were clipped uniformly during the summer of 1938 to control annual weeds. Notes were taken on the appearance of the plots. Wide differences between plots were noted in each species. Plots of Kentucky bluegrass, orchard grass, and white clover were in general well established with orchard grass plots making the most growth. A few plots of Canada bluegrass made a good growth, but this species was generally inferior, while timothy made the poorest showing.

Isolations from Permanent Pastures

The approximately 1500 sod plugs collected from permanent pastures in the Region in 1937 (See the 1937 Annual Report) were broken up in the spring of 1938 and one isolation of each species represented was taken from each sod plug. These isolations were planted in the field. The *Poa* species, particularly *Poa pratensis*, and *Trifolium repens* predominated. In addition, some plants of *Phleum pratense*, *Dactylis glomerata*, *Agrostis* spp., *Festuca* spp., *Lolium perenne*, and *Anthoxanthum odoratum* were obtained.

Preliminary observations indicate that approximately the same range of plant types were obtained from sod plugs as from seed collections. In *Trifolium repens*, however, there seemed to be less tendency to flower during the first summer among the sod plug isolations.

Physiology and Composition of Pasture Plants

The research activity relative to the composition of pasture plants and their response to certain environmental factors is still at the exploratory stage. At first the work was rather broad in nature but it is rapidly narrowing into more definite channels. The main lines of attack have been in methodology in both laboratory and greenhouse technique, and in studying the range in variation of pasture plants within a species, both as to composition and as to physiological response. The environmental conditions affecting reproduction have been studied with the object of speeding up the genetics program. A prime objective will continue to be the recognition of superior plants which may not be detected by morphological characters, but which may be detected by studies on drought resistance, temperature hardiness, growth and regenerative vigor, or nutritive value.

Fruiting as Influenced by Certain Environmental Factors

A preliminary experiment was set up to study the effect of length of day and temperature pre-treatments on the flowering of *Poa pratensis*, *P. compressa*, *Dactylis glomerata*, and *Phleum pratense*. On October 9, 1937, three plants of each species were selected from the field and brought into the greenhouse. Each plant was broken up into six pieces which were planted in a good soil in 4-inch pots. These were allowed to establish themselves in the greenhouse under natural day length.

On October 30, 1937, two pots from each clone were taken back to the field and buried in the soil to receive the normal temperature experienced under field conditions. One pot from each clone was returned to the greenhouse on December 11, 1937, at which time the soil was frozen to an average depth of 4 inches and was at a temperature of between -1.5 and -2.5°C. These plants will be designated as Lot II. On February 5, 1938, the one pot of each clone remaining in the field was returned to the greenhouse.

Table 13.- The effect of length of day and previous temperature treatments on heading of four grass species.

Species and clone	Number of heads per pot — 16-hour day: Lot I no cold treatment	Lot II field 42 days $-2^{\circ}C$.	Lot III cold room $7^{\circ}C$. 28 day	Lot IV cold room $0^{\circ}C$. 28 days	Lot V field 97 days $-5^{\circ}C$.	Natural day: Lot VI no cold treatment
Kentucky bluegrass						
KB 145(51)	0	2	3	3	6	0
KB 176(22)	0	0	0	0	7	0
KB 135(131)	0	0	0	0	4	0
Canada bluegrass						
CB 112(498)	26	18	26	26	21	0
CB 132(112)	19	22	33	10	20	0
CB 31 (33)	21	21	29	33	24	0
Orchard grass						
OG 1(14)	1	4	4	4	6	0
OG 1(18)	6	7	7	6	2	0
OG 13(9)	0	1	0	1	2	0
Timothy						
Ti 1(13)	6	6	5	3	3	0
Ti 6(5)	6	6	0	0	4	0
Ti 17(8)	0	7	0	0	1	0

The soil at this time had frozen to a depth of about 7 inches and had a temperature of about -6°C. These plants will be designated as Lot V.

On January 7, 1938, one pot of each clone which had been in the greenhouse since October 9 was placed in the cold room at +7°C. for 28 days, after which they were returned to the greenhouse. They will be designated as Lot III. Similarly one pot of each clone was placed in the cold room at 0°C. on January 7, 1938, for 28 days. These were returned to the greenhouse after 28 days and will be designated as Lot IV.

The two remaining pots of each clone were given no cold treatment, but were allowed to grow in the greenhouse at a temperature of 65°F. Beginning November 17, 1937, one pot of each clone (lot I) was given a 16-hour day with 100-watt Mazda lamps supplementing the natural day length. The remaining pot received only natural daylight at 65°F. and will be designated as Lot VI.

All the series which had received a cold treatment (Lots II to V) were also subjected to a 16-hour day beginning the day following their return to the greenhouse and the degree of heading was observed.

Due to mechanical difficulties with the cold rooms it was not possible to begin all lots to receive a 16-hour day at the same time so the detailed heading dates for each treatment will not be presented. When reproduction was initiated, the heads appeared from four to five weeks after the long-day treatment was begun. The results are summarized in table 13.

A 16-hour day in itself was not effective in producing flower stalks in the three clones of Kentucky bluegrass. One clone responded to each of the four cold treatments, +7°C. and 0°C. for four weeks, and buried in the soil for six and thirteen weeks. However, all three clones produced normal flowers and set good seed when they received a 16-hour day following a 13-week period in the field from October 30, 1937, to February 4, 1938.

Table 13.- The effect of length of day and previous temperature treatments on heading of four grass species.

Species and clone	Number of heads per pot: 16-hour day: Lot I no cold treatment	Lot II field 42 days -2°C.	Lot III cold room 7°C. 28 days	Lot IV cold room 0°C. 28 days	Lot V field 97 days -5°C.	Natural day: Lot VI no cold treatment
Kentucky bluegrass						
KB 145(51)	0	2	3	8	6	0
KB 176(22)	0	0	0	0	7	0
KB 135(131)	0	0	0	0	4	0
Canada bluegrass						
CB 112(498)	26	18	26	26	21	0
CB 132(112)	19	22	33	10	20	0
CB 31(33)	21	21	29	33	24	0
Orchard grass						
OG 1(14)	1	4	4	4	6	0
OG 1(18)	6	7	7	6	2	0
OG 13(9)	0	1	0	1	2	0
Timothy						
Ti 1(13)	6	6	5	3	3	0
Ti 6(5)	6	6	0	0	4	0
Ti 17(8)	0	7	0	0	1	0

A cold treatment did not appear to be necessary to induce flowering in Canada bluegrass, but a long day was essential inasmuch as no heads were produced on those pots receiving normal day length until late in the spring (April) when the normal day length had increased to 12 or 13 hours.

The orchard grass responded to increased day length with the production of heads which appeared to be favored by previous cold treatments. The results were not as positive as in the case of Kentucky bluegrass, but again it appears that a lower temperature (-5°C.) for a longer period favors head production.

Timothy responded to the 16-hour day in producing heads in two of the three clones. Low temperature pre-treatments in the field induced all clones to flower. It should be mentioned that while heads were produced in timothy about one-fourth of them produced aborted flowers and

few seeds. The failure to produce seeds may have been due to unfavorable growing conditions in the greenhouse.

Observations made on other material growing in the greenhouses during the winter of 1937-38 indicate that Canada bluegrass heads under a 16-hour day at 65°F., but that it is also stimulated to head under the short winter day when the temperature of the greenhouse is 70 - 75°F.

About two-thirds of the ninety-odd clones of Kentucky bluegrass, which were brought into the greenhouse for genetic work, headed without supplementary light in late February and early March, when the temperature of this greenhouse was maintained at 50°F. for several months prior to December 24, 1937 and then raised to 70°F.

In general, it appears that a long day and a preliminary cold treatment of several weeks at a temperature slightly below freezing induces heading of Kentucky bluegrass, orchard grass, and timothy. Canada bluegrass, however, appears to head satisfactorily when given a long day without a preliminary cold treatment.

Vernalization

Commercial lots of *Agrostis alba*, *Phalaris arundinacea*, *Dactylis glomerata*, *Poa pratensis*, *P. compressa*, and *Lolium perenne* seed were stored at two different temperatures for various lengths of time.

One hundred and fifty seeds of each species were placed on blotting paper in Petri dishes and moistened with 0.1 percent solution of potassium nitrate. The treatments given the seed may be outlined as follows:

7°C.	(Lot 1.	42 days not previously germinated
	(Lot 2.	21 days after germination at 25°C.
7°C. to 0°C.	(Lot 3.	21 days at 7°C. and 21 days at 0°C. after germination at 25°C.
0°C.	(Lot 4.	42 days after germination at 25°C.
	(Lot 5.	21 days after germination at 25°C.
	Lot 6.	No cold treatment

The seeds in all lots but No. 1 were germinated at 25°C. to a stage at which the primary roots were clearly visible and the shoots were one to mm. long before they were placed in the cold chambers. The blotters were maintained in a damp condition, but without excess water during the entire period of treatment.

The untreated Lot No. 6 was germinated at room temperature so that it could be transplanted to soil at the same time as all other series. The two 21-day lots (Nos. 2 and 5) had been started 21 days later than the two 42-day lots (Nos. 1 and 4), so that all could be taken from the cold chambers and transplanted on the same date. On April 1 all the seedlings were transplanted into soil in $1\frac{1}{2}$-inch paper bands in flats and placed in the greenhouse.

On May 3, 33 days later, several heads were coming out of the boot in the perennial ryegrass species, especially in those which had received a cold treatment of 0°C. for 42 days. On May 5, several Canada bluegrass plants were showing heads. Since the plants were in $1\frac{1}{2}$-inch paper bands, it was thought advisable to move these to the field. This was done on May 6. It was, however, unfortunate that they were transplanted at this time, because the dry period which followed delayed growth and when growth commenced it was from new basal buds.

On June 14 counts were made of the total number of heads developed on the various species. These are presented in table 14.

There was no indication of head formation on any plant of Kentucky bluegrass, reed canary grass, or red top on June 14, nor at any subsequent time during the summer. The plants in the several species which produced heads by June 14, did not produce additional heads during the course of the summer.

The data obtained in this experiment suggest that the vernalization treatment had little or no effect on timothy which normally flowers the

Table 14.- The number of heads produced on four species of grass following several vernalization treatments.

Treatment	Total No. of plants	Total No. of plants flowering	Number of heads produced	Average No. of heads per plant
Timothy-				
21 days, 7°, germinated	50	8	10	1.2
42 days, 7°, not germinated	7	1	1	1.0
21 days, 7°, 21 days, 0°, germinated	50	5	5	1.0
21 days, 0°, germinated	44	3	3	1.0
42 days, 0°, germinated	46	3	3	1.0
No cold treatment	50	8	8	1.0
Canada bluegrass-				
21 days, 7°, germinated	9	4	9	2.2
42 days, 7°, not germinated	4	2	6	3.0
21 days, 7°, 21 days 0°, germinated	27	15	32	2.1
21 days, 0°, germinated	5	1	2	2.0
42 days, 0°, germinated	24	15	31	2.1
No cold treatment	27	10	25	2.5
Perennial ryegrass-				
21 days, 7°, germinated	37	3	19	6.3
42 days, 7°, not germinated	39	8	33	4.1
21 days, 7°, 21 days, 0°, germinated	31	4	26	6.5
21 days, 0°, germinated	24	0	0	0.0
42 days, 0°, germinated	21	6	10	1.7
No cold treatment	20	0	0	0.0
Orchard grass-				
21 days, 7°, germinated	30	0	0	0.0
42 days, 7°, not germinated	44	1	1	1.1
21 days, 7°, 21 days 0°, germinated	49	0	0	0.0
21 days, 0°, germinated	14	0	0	0.0
42 days, 0°, germinated	23	2	2	1.0
No cold treatment	25	0	0	0.0

first year in the field. In Canada bluegrass the cold treatment did not increase the total number of plants flowering, nor the number of heads produced. They did, however, bring the plants into flowering much earlier, since heads appeared only 35 days after planting.

Perennial ryegrass, which may normally produce a small number of flowers the first year in the field, seemed to show the greatest response to the vernalization treatment. The longer, 42-day, treatment appeared

to be more effective than the shorter, 21-day, treatment, although the data is insufficient to be conclusive. The period from planting to heading (32 days) is very much shortened, which may be of advantage in a breeding program.

This preliminary experiment suggests that following a vernalization treatment, Canada bluegrass and perennial ryegrass can be forced into heading 30 to 35 days after planting.

Water Relations

Fertility and defoliation have been shown to affect the water requirements of plants. An experiment was set up to study the water utilization of Poa pratensis as it is affected by nitrogen fertility and clipping treatments.

Clonal isolations from four plants of widely divergent morphological types were used: (1) narrow leaf, erect; (2) wide leaf, erect; (3) narrow leaf, decumbant; and (4) wide leaf, decumbent.

On October 7, 1938, nine single tillers from each clone were started in automatic gravel cultures (described under Culture Methods) supplied with a balanced nutrient solution.

The plants were allowed to grow until December 8, 1938, when all were cut at the one-inch level. The nutrient solutions were changed on this date. One-half of the pots of each clone received a high nitrogen nutrient and the remainder received a low nitrogen nutrient. The bottles containing the nutrient solutions were filled with distilled water to replace that lost by evaporation and transpiration at intervals of several days. The amount of water added to each bottle was recorded. The evaporation from the gravel surface was determined from gravel cultures with plants distributed at random on the benches.

Beginning December 8, 1938, clipping treatments, varying in both frequency and height of cut, were given to each clone.

To date, insufficient data has been obtained to warrant reporting.

Nutrient Culture Methods

The apparatus here described can be easily installed on any bench and has proved very satisfactory during the past 12 months. It is compact and a culture may be easily added or removed. It has the added advantage that the air under low pressure can be easily taken to various parts of the greenhouse through glass tubing thereby making it possible to change the arrangement of any of the cultures at will.

In principle, the irrigation of the gravel cultures depends upon air under low pressure to force the nutrient solutions from airtight storage containers up into jars. When the air pressure is released the solution returns to the containers by gravity, and at the same time aerates the cultures.

One-gallon glazed jars, having a glazed hole flush with the bottom, were used throughout. The nutrient enters the jar through a 2-mm. capillary tube in a rubber stopper at the bottom. A pad of glass wool over the end of the stopper on the inside of the jar retains the gravel. A watch glass was placed over the glass wool to prevent packing.

It was found equally satisfactory to use a large carboy of 20 liters or more capacity to supply 10 pots with the same nutrient or to supply each culture independently from 2-liter nutrient bottles. These were connected to the compressed air supply shown in figure 2, A.

When a large carboy was used as a storage tank for the nutrient (see figure 2, B), a 12-mm. glass tube delivered the nutrient to an inverted 250 cc. glass bottle with bottom cut off. A size 10 rubber stopper with 7 holes, 5 for outlet tubes to the jars, one for introducing nutrients

and water lost through transpiration and evaporation when the concentration of any element is to be maintained daily, and one small outlet with rubber tube and pinch cock for sampling the nutrient at the time it is being delivered to the cultures. The five outlet tubes to the jars are connected with rubber tubing to Y tubes so that each tube from the carboy supplies two jars. The air inlet to the carboy from the air supply line has a 1-mm. glass capillary tube inserted to regulate the air flow between the various carboys and in case of accident to one carboy, the remainder would not be seriously affected.

The individual cultures are connected to the air supply in series by means of T tubes, as shown in Figure 2, B. Ten to twelve cultures in a series have proved satisfactory. The air inlet to each series is regulated by a 1-mm. glass capillary to give a uniform pressure to a number of series. The nutrient to each jar is supplied by a 4-mm. glass tube extending to the bottom of the 2-liter bottle. The individual bottles are painted black to cut out the light and prevent algal growth and are subsequently enameled white to reflect light and keep the solution from becoming excessively warm. The large reservoirs or carboys may also be painted or may be protected from light by tightly fitted dark window shades.

Where a constant source of compressed air is not available, compressed air may be supplied by means of a small electric pump with pressure regulator. The pump is turned on at the desired times and for the proper duration (usually ten minutes) by means of a time clock and relay. An open-end U tube filled with mercury is placed in the air line to indicate the pressure in the system. The pressure delivered by the pump is regulated so that during the interval for which the clock is set, the nutrient will be raised to about one-half inch below the surface of the gravel. If it should go higher no harm will result, since the jars are filled only to

within three-quarters inch of the top which is an ample reservoir for slight variation in air pressure or differences between jars after the correct air pressure is established. If the surface of the gravel is irrigated continuously, however, algal growth in the top becomes objectionable.

It has been found desirable to irrigate the cultures once every three hours during the summer and once every six hours during the winter when transpiration is not so rapid. The water lost from the individual 2-liter bottles and carboys is replaced daily with distilled water and the nutrient solutions discarded and replaced with fresh nutrient every two weeks.

Growth and Composition of *Poa* Grown in Nutrient Cultures

Preliminary to a study of reserve substances and their role in growth, *Poa compressa* and *P. pratensis* were grown in sand cultures and sampled for chemical analysis. Some analyses have been made. Further analyses will be made after further study on carbohydrate methods.

Clonal isolations from a single plant of Canada bluegrass were grown under 13 variations in environmental conditions. Early growth was more rapid with high and medium nitrate nutrient than with low nitrate nutrient. It was more rapid with medium nutrient concentration ($1\frac{1}{2}$ atmospheres) than with low nutrient concentration ($\frac{1}{2}$ atmosphere) and especially more rapid than with high nutrient concentration (3 atmospheres). Growth was more rapid with a long day (16 hours) than with a natural day-length (January to June), and at a higher temperature (70°) than at a lower temperature (65°). The plants grown under low light intensity, obtained by shading with cheesecloth, grew taller than those receiving full light when grown at the higher temperature but not when grown at the lower temperature.

Flower buds were first detected in the long-day plants with high nitrate nutrient and at the higher temperature. Later, heads were produced abundantly on all the long day plants, particularly on those plants receiving

the higher nitrate and the lower concentration nutrients. All plants flowered to some extent. The slightly shaded plants produced a considerably less number of heads than plants receiving full daylight.

The factors which tended to produce the greatest yields of tops and roots were high and medium nitrate nutrients, long days, higher temperatures, and high light intensity. Soluble nitrogen was higher in the high and medium nitrate plants than in the low nitrate plants. The roots from long-day plants contained less soluble nitrogen than roots from the short-day plants receiving otherwise similar treatment. The plants grown under high concentration nutrients produced the least amount of dry matter but usually contained more soluble nitrogen than those grown under the lower concentration nutrients.

When plants were clipped periodically the dry weights of clippings progressively decreased. The plants grown under high nitrate conditions recovered rapidly from the first clipping but with subsequent clippings they recovered more slowly than the low nitrate plants and died sooner. The high concentration nutrient series died after a few clippings. The low nitrate short-day plants were alive after seven clippings, and the total weight of clippings produced was greater than with any other series. In general, the total nitrogen content of the clippings decreased in the successive clippings.

Kentucky bluegrass was grown for 6 weeks under eight different nutrient treatments and then clipped to ground level. Plants that had been grown with medium nitrate and with low nitrate nutrient produced more new growth than those grown with high nitrate nutrient. There was less new growth produced from the plants receiving high concentration nutrient than from those receiving the low. Plants supplied with a nutrient solution low in potassium showed especially good growth and recovery.

Differential Responses of Clones of *Trifolium repens* and *Poa pratensis* to Different Levels of Soil Acidity and Fertility

Inherent differences in the response of 29 clones of *Trifolium repens* to different levels of soil acidity and fertility were investigated in the greenhouse. These 29 clones were selected to represent the more striking morphological differences found in the white clover nursery. Each clone was grown in duplicate in 1-gallon pots of Dekalb silt loam with seven levels of mineral nutrition. The foliage was removed at intervals by plucking, and the yields of dry matter determined.

Table 15 gives data on the average yields obtained and on the individual yields of a few selected clones.

Table 15.- Yields of different clones of white clover at various levels of soil acidity and fertility.[1]

Clone No.	Total yield of 4 pluckings (grams per pot)						
	None	L	P	P-L	2P-L	P-2L	2P-2L
Average of 29 clones	2.0	3.4	5.3	10.8	14.5	12.6	18.8
44(23)	3.1	5.8	8.8	16.6	22.8	19.7	27.0
46(76)	0.8	1.9	3.8	9.5	11.4	9.9	18.2
33(37)	1.3	2.9	4.4	9.5	11.2	8.5	12.2
15(31)	1.8	2.7	4.8	10.4	14.6	13.7	22.3
57(141)	2.3	4.3	5.3	12.0	13.1	11.0	16.5

[1] The untreated soil was strongly acid (PH 4.9) and very low in available phosphorus (4 pounds per acre by the Truog method). The L and 2L treatments represent PH values of 5.6 and 6.4, respectively. The P and 2P treatments were 50 and 100 pounds per acre of P_2O_5, respectively. All pots received potash.

The average for all clones shows very low yields on the untreated soil but good response to both lime and phosphorus. Clone 44(23) was outstanding from the standpoint of yield, producing about 60 percent more than the average. Clone 46(76) was the lowest producer at the low

fertility levels, but gave about aveage yields at the highest fertility level. No. 33(37) responded well to the first increment of lime and phosphorus but gave relatively little additional increase at the high fertility level. Thus, at the low fertility levels (none, L, and P) clone 46(76) yielded less than clone 33(37); at a somewhat higher level (P-L, 2P-L, and P-2L) the yields of the two clones were about the same; but at the highest nutrient level 46(76) yielded nearly 50 percent more than 33(37). Clone 15(31) also responded well to heavy fertilization, whereas 57(141) did not.

A period of hot dry weather the first of August, immediately following the third plucking, resulted in partial killing of some clones, particularly in the pots of lower fertility. Because of this injury, together with subsequent injury from red spider mite, the fourth plucking was discarded. As shown in table 16 the resistance of a clone to injury was associated with the size of the stolons. The five clones with the thickest stolons were not injured, whereas 75 percent of the others were injured.

Table 16.- Relation between injury to hot, dry weather and the relative thickness of the stolons in white clover.

Relative estimated diameter of stolon	Degree of injury				
	None	Slight	Medium	Severe	Very severe
Large	3	0	0	0	0
Medium-large	2	0	0	0	0
Medium	3	3	4	0	0
Medium-small	1	4	0	0	0
Small	2	3	2	1	1

Studies similar to those described for white clover have been started with Kentucky bluegrass. These preliminary studies with white clover and Kentucky bluegrass show the importance of considering the fertility level of the soil when evaluating different clones. Clones that produce

superior yields at one level of soil fertility may not produce superior yields at some other fertility level.

Hydrocyanic Acid Content of *Trifolium repens*

During 1938 the work on HCN in white clover consisted of a survey to determine the range in concentration of this substance and to select plants high in HCN for further study.

During the winter 66 plants were available in the greenhouse. They were analyzed by the Boyd method with the results shown in table 17.

Table 17.- Range in HCN content of white clover grown in the greenhouse.

Number of Plants	HCN in p.p.m. of fresh weight
41	0-10
12	11-50
8	51-100
5	over 100

The highest figure obtained was 320 p.p.m. (37W62(177)). Two-thirds of the plants contained practically no HCN. Ten p.p.m. could not be readily distinguished from 0 p.p.m. No morphological characters were found to be common to those plants high in HCN.

Further studies were carried out during the summer on field material between August 3 and August 16. Individual plants from the nursery were tested by the qualitative method of Rogers. When a positive test was obtained the plant was tested by the quantitative method of Boyd. The 66 plants tested during the winter were included in these tests and in only one case was a discrepancy found. The results are given in table 18.

Table 18.- Range of HCN content of white clover in the field, August 1938.

Number of plants	p.p.m.
194	0-10
9	11-50
15	51-100
19	101-200
9	201-300
4	over 300
13	more than 10 but not tested quantitatively
Total 263	

Those plants in the field from seed lot 62 (originating in Michigan) showed a greater number of positive plants. On August 18, 62 additional plants, making a total of 75, from seed lot 62 were tested qualitatively. Only 8 plants gave a negative test. The relation between the more vigorous plants and the intensity of the color test is of questionable significance. ($r = .34 \pm .11$).

Seed lot 41, which originated in Lennoxville, Quebec, was similarly studied. On August 20 60 plants from this lot were selected from a seemingly uniform soil area; 5 x 12 rows. Of these only 21 gave positive tests and since 4 of these gave very faint tests they were included in the 42 plants classed as negative. The data are summarized in table 19.

Table 19.- Summary of HCN analyses.

	No. tested	Negative plants	Percent negative
Seed lot 62	75	8	10.7
Seed lot 41	60	43	71.7
Field as a whole	263	194	73.8
Greenhouse	66	41	62.1

Progeny from inbred plants were available for study. The results appear in table 20.

Table 20.- HCN content of parents and progeny.

	Parent testing	Number of progeny	Number positive	Number negative
Inbred 3	+	30	30	0
Inbred 4	+	29	29	0
Inbred 15(31)	-	17	0	17

The highest HCN content (500 p.p.m.) was found in the most vigorous plant in the field. This plant, 37W47(86), was grazed by a sheep about August 18 and had no ill effects on the animal. The new growth which followed was lower in HCN than the previous growth which had been grazed. However, rain had fallen in the meantime and since the previous samples had been taken during extremely dry weather, some of the plants high in HCN were reanalyzed. Lower results were obtained in all cases as shown in table 21.

Table 21.- Variation in HCN content on two different dates.

Plant No.	Dry period		Period following rain	
	Date	p.p.m.	Date	p.p.m.
46(76)	8/4	170	9/15	40
47(86)	8/15	500	9/15	240
49(55)	8/4	220	9/15	100
62(20)	8/16	300	9/14	120
62(71)	8/16	260	9/14	90
62(177)	8/4	280	9/15	110

The analytical methods followed are those which have been used by workers on Sudan grass. In view of the fact that one of the plants which was high in HCN was not toxic in one feeding trial, the possibility that the analytical methods are not reliable may exist. An alternative view is that the glucoside containing HCN requires a highly specific enzyme which was not present in the animal used.

A preliminary attempt to isolate the glucoside was made. An alcoholic extract of white clover leaves yielded, after clarification, a protein-free residue from which HCN was liberated readily by takadiastase but not by dilute acid or warm water.

Preliminary tests indicate that about one-fourth of all white clover plants contain variable amounts of hydrocyanic acid. In a few plants the concentration was high but toxicity to animals has not yet been demonstrated.

Variation in Crude Protein of *Poa pratensis*

This problem was undertaken for the purpose of determining if, and to what extent, variation existed in the chemical composition of plants within a species. Because of the simplicity of the crude protein determination (total nitrogen) this constituent was studied first.

To avoid soil variation as much as possible, since replications did not exist, a spot was chosen in the nursery, about 20 x 40 feet, which appeared to be uniform in soil type and in slope. In this area one hundred and thirty-nine one-year old plants from open-pollinated seed were selected for study.

Since the relative stage of growth determines to a considerable extent the chemical composition of a plant, it was necessary to work with plants of uniform maturity. Because the degree of maturity is most readily recognized at the flowering stage the plants to be studied were selected when in full bloom. Three degrees of maturity were recognized, early, medium, and late, and the plants were tagged accordingly. All the plants selected bloomed within a period of one week.

The plants were sampled for analysis on May 27, the same day they were tagged. They were cut at about one inch above ground level and hung in paper sacks in the greenhouse to dry. All brown tissue was discarded and the remainder divided into three classes: culms, leaves, and heads.

The culms and leaves were analyzed for total nitrogen. The results are expressed as percentage of total nitrogen of the oven dry weight.

After sampling the plants were mowed with a field mower. Collections of the aftermath were made on August 25. The analytical results are presented in table 22.

Table 22.- Percentage of total nitrogen in Kentucky bluegrass.

Group depending on time of flowering	No. of plants in group		Percentage of total nitrogen May 27 Leaves	Percentage of total nitrogen May 27 Culms	Percentage of total nitrogen August 25 Leaves
Early	39	Max.	2.50	1.18	3.03
		Min.	1.38	.74	1.89
		Mean	2.02	1.01	2.47
		St.dev.	.23	.09	.22
Medium	75	Max.	2.60	1.33	3.01
		Min.	1.44	.92	1.99
		Mean	2.10	1.09	2.58
		St.dev.	.22	.10	.19
Late	25	Max.	2.53	1.65	3.07
		Min.	1.64	.90	1.92
		Mean	2.12	1.27	2.64
		St.dev.	.19	.19	.25
Total	139	Max.	2.60	1.65	3.07
		Min.	1.44	.74	1.89
		Mean	2.08	1.10	2.56
		St.dev.	.20	.14	.22

A progressive increase in the mean nitrogen content of the plants from the early to the late group was present at both dates. In the harvested leaves on May 27 there is no significant difference between the early and medium groups, nor between the medium and late groups. There is a slight

significant difference between all groups of culms harvested on May 27. The August leaves harvested August 25 show significant differences between medium and late groups.

There is nothing surprising in the increase of total nitrogen on passing from the early to the late groups in May as the leaves and culms at this time were rapidly losing nitrogen to the developing heads and the earlier groups had progressed further in this direction. The August samples were composed of second growth and the plants were not heading; no differences were expected to be found between groups which were defined during the flowering stage three months before, but such differences did exist.

No relation appeared to be present between the nitrogen content of a plant and its location in the plot. There was a random distribution of plants above and below the average of its group.

It was of particular interest to determine whether plants high or low in nitrogen at one period showed a similar content at another period. A significant correlation existed between the leaves and culms harvested on the same date. Correlations also existed between samples harvested in May and those harvested in August. A greater correlation was found, however, between the leaves sampled in August and the culms harvested in May than between the leaveson the two dates. The results are shown in table 23.

Table 23.- Correlation coefficients between samples.

Group	No. of plants	Leaves and culms of May	Leaves of May and leaves of August	Culms of May and leaves of August
Early	39	.57	.08	.48
Medium	75	.81	.34	.36
Late	25	.83	.63	.72
Total	139	.65	.34	.54

The first year's study indicates that a considerable range in total nitrogen content exists in the foliar portions of Kentucky bluegrass.

Improvement in Chemical Methods of Analysis

Work is in progress which has for its aims the identification of the carbohydrates present in various grass species, their quantitative separation and analysis. Using leaves of Poa compressa as the experimental material, a method of hydrolyzing fructosans with oxalic acid was studied by varying the temperature, concentration of acid, and duration of heating. Both glucoside and fructose were determined in the products of hydrolysis. As the conditions of hydrolysis became more drastic the total reducing power of the hydrolyzate increased but fructose, after reaching a maximum, did not further increase. This may indicate that fractions more resistant to hydrolysis contain relatively more glucose than fructose.

A better method of measuring glucose and fructose in mixtures seemed desirable. The method of destroying the reducing power of glucose by oxidizing it to gluconic acid by means of iodine in alkaline solution and the subsequent determination of the residual reducing power as fructose was studied. The addition of 4 cc. of 15 percent Na_2CO_3 and sufficient iodine completely oxidized glucose in 2 hours and did not affect fructose. Lower concentrations of Na_2CO_3 or various concentrations of borax did not allow complete oxidation of glucose.

An 80 percent alcoholic extract of the underground parts of the same species was studied with various hydrolytic agents. The results indicate that sucrose is the only substance present with undergoes hydrolysis, and that alcoholic extraction may quantitatively separate sucrose from other more complex fructosans.

The concentration of salts present in solution following the oxidation of glucose affects the determination of the residual fructose. NaCl,

Na_2SO_4, sodium oxalate, KI, and to some extent sodium acetate decrdase the reducing power of sugar when a copper-carbonate micro method is used. Potassium iodide alone affects a more alkaline macro copper method by increasing the reduction.

The accurace of the micro sugar method was also improved by adding a small amount of aluminum hydroxide before centrifuging the reduced copper. Loss was thus prevented when the supernatant liquid was decanted.

These studies in the improvement of chemical methods dealt primarily with the carbohydrates of grasses.

Germination Studies

Kentucky bluegrass

The prompt germination of freshly harvested *Poa pratensis* seed is desirable in a cytogenetic or breeding program where several generations are to be grown in one year. Seed harvested in March, 1938, from a number of plants of Poa pratensis had a germination of only 0 to 10 percent when placed at room temperatures 20° to 24°C.). Several experiments were conducted to determine the environmental treatments which were effective in breaking the dormancy of these seeds.

On June 21, 1938 seed heads were harvested from six plants in the field. Samples of seed were taken from each for the determination of moisture. Three samples contained moisture ranging from 11.8 percent to 14.6 percent, while the three remaining had a moisture content between 23.5 percent and 32.9 percent. The remainder of the seed was air dried and threshed for use in germination tests which involved the storage of the dried seed at several temperatures from -7°C. to +43°C. for a total of 64 days. Samples were taken for germination at the beginning of the experiment and after 4, 8, 16, 32, and 64 days, fifty plump seeds of each of the six lots were placed on blotting paper moistened with .1 percent

KNO_3 in Petri dishes and allowed to germinate on the laboratory table at room temperature (22° - 26°C.) for 4 weeks, after which no further germination took place.

An examination of the data presented in table 24 demonstrates rather conclusively that the various storage temperatures used (from -7°C. to +43°C.) were without effect on breaking the dormancy of the dry seed. It will be noticed that after 32 days' storage at any temperature, the dormancy of the seed was breaking with a resultant increase in germination percentage to between 20 and 30 percent and that after 64 days' storage, the germination had reached to between 20 and 50 percent.

Table 24.- Average percent germination of freshly harvested seed from six plants of Kentucky bluegrass.

Treatment of germinating seed	Number of days of storage of dry seed at indicated temperatures					
	0	4	8	16	32	64
Room temp. 22° - 26°C.						
None	1.0	- -	- - -	- -	- -	36.7
Cold treatment (moist)[1]	79.0	- -	- -	- -	- -	91.6
Room temp. 43°C.						
None	- -	0.7	1.0	2.7	1.3	51.7
Cold treatment (moist)[1]	- -	91,0	89.0	88.7	84.7	90.3
Room Temp. 0°C.						
None	- -	0.3	0.7	1.0	27.3	22.3
Cold treatment (moist)[1]	- -	84.0	91.0	91.0	89.2	89.3
Room temp. -7°C.						
None	- -	0.3	1.7	1.3	31.0	25.0
Cold treatment (moist)[1]	- -	90.6	87.7	90.0	90.4	90.6

1/ After 4 weeks germination at room temperature the Petri dishes with ungerminated seed were placed at +7°C. for 7 days and then returned to the laboratory for subsequent germination.

The cold treatment of the moist seed, which had a low germination at room temperature, initiated a practically normal germination of this seed when returned to the laboratory, bringing the total germination to between 88 and 90 percent. This final germination did not appear to be affected by the previous storage temperatures or the length of time which the dry seed

was stored.

A second collection of seed heads was made from the same plants (July 5, 1938). The stems as well as the heads were brown and the percentage moisture in the threshed seed ranged between 10 and 11 percent.

Instead of treatment of dry seed, various treatments were tried on moistened seeds. The seed was threshed and 50 seed-samples of plump seed (all withered or small seeds were discarded) were counted out onto blotters in Petri dishes. The blotters were moistened with .1 percent KNO_3 and allowed to remain in the laboratory at room temperature for 24 hours. At the end of this time the seed had imbibed water and the seeds were fully turgid. One lot, including a 50-seed sample from each plant, was placed in a constant temperature chamber at 43°C., one lot at +7°C., one lot at 0°C., one lot at -5°C., and one lot was left at room temperature in the laboratory. After being allowed to remain for 7 days at their respective temperatures all lots were brought back to the laboratory for germination. The results of this test are summarized in table 25.

Table 25.- Average percent germination of freshly harvested Kentucky bluegrass seed from six individual plants.

Treatment	Germinated at room temperature	After transfer at 7°C.
Room temperature	0	94.3
7 days at 43°C.	0	93.3
7 days at +7°C.	77.4	92.0
7 days at 0°C.	39.3	95.7
7 days at -5°C.	0	83.3

It will be observed that after 4 weeks there was no germination of any of the seed which had been at room temperature, 43°C., or at -5°C. There was an average of 77.4 percent germination in the seed receiving +7°C. and 39.3 percent in the seed at 0°C. for 7 days. After the 4 weeks' germination period in the laboratory at room temperature, all lots were

placed at +7°C. for an additional 7 days after which they were brought back to the laboratory. This increased the germination of all lots of seed to between 92 and 95 percent, except the lot which had received the previous -5°C. This lot germinated only 83 percent, which may have been due to cold injury occasioned by the first treatment.

The results of these experiments indicate that none of the temperatures used (-7°C to 72°C.) on dry seed affected an immediate break in dormancy. Treatment of the moistened seed, however, at +7°C. for 7 days increased germination from 0 to 77.4 percent and similar treatment at 0°C. increased the germination from 0 to 39.4 percent. The optimum temperature and length of time to cause the break in dormancy has not yet been determined.

Dormancy is not caused by seed coats impermeable to water, inasmuch as all seed readily imbibed water whether they germinated or not. The stage of harvest or percent of moisture in the seed did not affect the ability of the seed to germinate.

Orchard Grass

The seed from two plants of *Dactylis glomerata* growing in the greenhouse was harvested March 24, 1938. The heads were brown but the stems were slightly green and the seeds were soft. Twenty-five threshed seeds were taken from each plant and germinated as before at room temperature. The remaining heads of each plant were divided into three groups, two of which were threshed and the other remained intact. Samples of threshed seed and the unthreshed heads were placed in an oven at 43°C. to after-ripen. The remaining threshed seed was allowed to after-ripen at room temperature. Samples of the seed in the oven and at room temperature were taken out for germination after 3, 6, 12, and 25 days. The results of the test are presented in table 26.

Table 26.- The percent germination of orchard grass seed stored at various temperatures for different lengths of time.

Plant No.	Days in oven at 43°C.								Days at room temperature				Immediate germination
	Threshed				In head								
	3	6	12	24	3	6	12	24	3	6	12	24	
37#0g48(136)	96	88	100	64	88	100	100	76	80	88	100	88	76
37#0g48(270)	100	96	100	92	--	100	--	92	100	100	100	96	100

These data suggest that plant No. 37#0g48(270) produces seed of higher germination than plant No. 37#0g48(136). It appears that a short after-ripening period of 3 to 12 days at room temperature or in the oven at 43°C. will slightly increase germination. Twenty-four days at 43°C. tends to reduce germination. Even at the end of the 12-day period, those seeds stored at room temperature produced the more vigorous seedlings.

Some seeds in which the caryopsis had been separated from the glumes were tested. Such seeds germinated perfectly and appeared to germinate sooner than the normally glume-enclosed seeds.

Vegetative Propagation of *Poa pratensis* and *P. compressa*

Perennial ryegrass species have an advantage over many annual species in offering a means for carrying on and increasing a single genotype by clonal propagation. Since the rapidity with which any one plant may be increased is of considerable importance an experiment was set up to determine the size and number of clonal isolations which could be obtained from an average-sized plant. One year old plants of *Poa pratensis* and *P. compressa* were taken from the field into the greenhouse on November 20, 1937. About one-third of each plant was used in this experiment. The plants were broken up into different sized pieces, the largest consisting of 3 or 4 tillers each with tall vigorous leaves and the smallest piece consisting of but a single tiller devoid of chlorophyl and coming from a rhizome.

One half of the slips from each species was treated with a .03 percent solution of auxilin (indoleacetic acid) for 24 hours prior to planting; the other half was treated with tap water for the same period. The cuttings of each species and treatment were divided according to size into six classes in the case of Kentucky bluegrass, and into four classes in the case of Canada bluegrass. One-third of the cuttings in each class was planted in 3-inch clay pots of river bottom send, one-third in a loam soil, and the remainder in well-rotted manure. The number of pots in each treatment varied between 1 and 7, with 4 or 5 pots in most classes. The pots were placed in the greenhouse and watered regularly with tap water. They were allowed to grow without further treatment, except for watering, for 90 days, when the number of tillers and the height of the tallest leaf in each pot were recorded.

The data for Kentucky bluegrass are presented in table 27 and those for Canada bluegrass in table 28. Inasmuch as the auxilin treated cuttings

Table 27.- Number of tillers and height of tallest leaf produced by Kentucky bluegrass cuttings after 90 days growth in three media.

Class No. 1/	Average number of tillers per pot				Average height in cm. of tallest leaf per pot			
	Sand	Soil	Manure	Average	Sand	Soil	Manure	Average
1	10.0	- -	12.0	11.0	10.0	- -	12.0	11.0
2	9.0	13.2	15.8	12.7	8.9	10.7	12.7	10.8
3	5.2	9.0	10.6	8.3	7.3	9.7	14.6	10.5
4	5.7	10.1	13.2	9.7	8.4	9.5	11.8	9.9
5	9.0	10.5	16.5	12.0	7.6	8.2	10.8	8.9
6	7.7	9.1	17.5	11.4	7.3	8.5	10.2	8.7
Average	7.8	10.4	14.3	- -	8.2	9.3	12.0	- -

1/ Key to classes-
1. Green leaves, 3" to 4" tall, 2, 3, or 4 tillers to a pot.
2. Green leaves, 2" to 3" tall, 3, 4, or 5 tillers to a pot.
3. Green leaves, 2" to 3" tall, 1 or 2 tillers to a pot.
4. Tillers or branches from rhizome, tips of leaves green, plants not well formed.
5. Tillers or branches from rhizome, devoid of chlorophyll.
6. Similar to class 5, but only 1/2 as large.

Table 28. Number of tillers and height of tallest leaf produced by Canada bluegrass cuttings after 90 days growth in three media.

Class No. 1/	Average number of tillers per pot				Average height in cm. of tallest leaf per pot			
	Sand	Soil	Manure	Average	Sand	Soil	Manure	Average
1	10.3	13.4	15.7	13.1	12.6	14.6	14.4	13.9
2	7.7	8.0	13.1	9.6	10.6	15.1	14.7	13.5
3	6.8	11.6	12.9	10.4	11.4	15.3	15.3	14.0
4	4.0	6.0	15.0	8.3	12.0	9.0	13.5	11.5
Average	7.2	9.7	14.2	- -	11.7	13.5	14.5	- -

1/ Key to classes-

1. Single green tiller, 4" to 6" long; short buds at base.
2. Single green tiller, no buds at base.
3. Single tillers 3" to 5" long, branch from rhizomes, devoid of chlorophyll or only tips green.
4. Single tillers 1" to 3" long, branch from rhizome, devoid of, or with only slight amount of, chlorophyll.

were not significantly different from the untreated, the data from these two treatments have been averaged. Inasmuch as the Kentucky and Canada bluegrass exhibit similar responses, they will be spoken of jointly. The size of the clonal piece used for starting a new plant did not materially affect the size of the plant or the number of tillers after 90 days of growth. In some instances the larger pieces used produced slightly larger plants with a few more tillers.

The greatest differences were found between the different mediums used in the pots. Those cuttings in sand gave the increase in size of plant and number of tillers, due, no doubt, to lack of nutrients. Those in soil showed favorable increases over the sand. The well-rotted manure, however, appeared to be the best medium in which to plant slips or cuttings for clonal increase.

It is estimated that the total number of plants which could have been obtained by cuttings from each of the one-year-old plants used in this experiment is over 500 in the Kentucky bluegrass and 400 in the Canada bluegrass.

Several rhizomes without terminal buds were planted in sand and soil. These produced branches at nearly every node. No measurements were made on this material. The auxilin-treated cuttings rooted somewhat earlier than the untreated cuttings but after 90 days showed no advantage over the untreated, either in size of plant or number of tillers present.

These data indicate that the available nutrient supply is more important than the size of cuttings used in obtaining clonal increases of Kentucky and Canada bluegrass, and that several hundred clonal isolations may be obtained from a single year-old field-grown plant.

Methodology in Transplanting Grasses

Methods of starting seedlings and cuttings in the greenhouse and transplanting them to the field were investigated in a preliminary manner. Seedlings of *Poa pratensis*, *Agrostis alba*, and *Phleum pratense* were used and the following treatments given:

1. Planted in 1-inch bands in flats, the bands not removed when transplanted.
2. Planted in 1-inch bands in flats and the bands removed when transplanted.
3. Planted in 2-inch bands in flats, the bands not removed when transplanted.
4. Planted in 2-inch bands in flats, the bands removed when transplanted.

For each species, four 4 x 4 latin squares were used. In taking notes, the number of missing plants was recorded and the survivors were graded from 1 to 4. Due to missing plants, statistical analysis did not seem advisable. Little difference was noted between treatments 1, 3, and 4, but treatment 2 was inferior.

For studies of technic of handling clonal material, *Poa pratensis*, *Dactylis glomerata*, *Phleum pratense*, and *Agrostis alba* were used and the treatments were as follows:

1. Planted 1 inch apart without bands in flats.

2. Planted in 1-inch bands in flats, the bands not removed when transplanted.

3. Planted in 1-inch bands in flats, the bands removed when transplanted.

4. Transplanted directly to the field.

Noted were taken in the same manner as described for the seedlings. No consistent differences were noted in the different treatments. Under the conditions of the experiment the only consistent difference observed was the deleterious effect of removing the 1-inch bands before transplanting seedlings to the field.

Methodology in Transplanting Legumes

The results with seven clones of white clover planted in eight replications, arranged in two latin squares are shown in table 29. Plants were scored on vigor on the basis of 1-10.

Table 29.- Survival of white clover clones.

Treatments	Average percent of survival	Average vigor of survivors
$1\frac{1}{2}$-inch band - taken off at planting	95	6.1
$1\frac{1}{2}$-inch band - left on at planting	89	5.9
Spaced $1\frac{1}{2}$ inches each way in flats without bands	73	5.0
Planted directly in field	32	3.4

Four treatments using $1\frac{1}{2}$- and 2-inch bands which were either taken off or left on at planting were tried on seedlings of white clover with 24 replications of each treatment arranged in six latin squares. No significant differences in either percent of survival or vigor of survivors were observed.

In transplanting clonal material of white clover from the greenhouse to the field, it has been demonstrated that best results may be expected by first starting the clonal pieces in flats rather than taking them directly to the field.

Changes in Soil Phosphorus During Storage

Marked changes in acid soluble phosphorus may occur in air-dry pasture soils during storage. For a preliminary investigation of conditions effecting these changes a sample of Dekalb silt loam from the surface 2-4 inches of a highly productive area in a Kentucky bluegrass-white clover pasture was brought to the Laboratory in March, 1938. It was screened and well mixed when still very moist. Portions were treated in various ways and available phosphorus determined at intervals by the Truog method.

Table 30 summarizes the effect of different methods of preparing the soil on the values obtained for available phosphorus. Samples stored moist for 8 days showed an 18 percent reduction in readily available phosphorus, but, upon drying, the available phosphorus content increased to its original value. When weighing out samples of moist soil for analysis, allowance was made for the water content. Careful trails showed that in this soil the water present in moist samples did not dilute the extracting solution enough to affect the results.

The changes in available soil phosphorus during storage in 4-ounce glass bottles are shown in table 31. The available phosphorus of samples stored in the laboratory for 9 months increased 34 percent while samples stored at about 5°C. increased 23 percent. These changes during storage appeared to be independent of the various methods employed in drying the sample, i.e., air-dried, oven-dried, and moistened for 1-5 weeks and then air-dried. The data also indicate that the increase in phosphorus solubility during storage was a gradual process.

Table 30.- The effect of preliminary drying treatment on the available phosphorus content of a Dekalb silt loam as measured by the Truog method.

Treatment of soil sample 1/	Available phosphorus (pounds per acre)
Air-dried immediately after screening	90
Analyzed before drying	85
Kept moist in a stoppered bottle for 8 days after screening	74 2/
Kept moist in an open bottle for 8 days after screening	73 2/
Moist for 8 days and then air-dried	90
Air-dried then kept moist 5 weeks (not stoppered)	80 2/
Air-dried then moist for 5 weeks and air-dried	96 3/
Air-dried then dried 5 hours in vacuum oven at 35°C.	93
Air-dried then dried 5 hours in an ordinary oven at 100°C.	104 2/

1/ All treatments were in quadruplicate.

2/ Only these values are significantly different from the value obtained for the air-dried sample.

3/ This value agrees very well with the value of 97 for the soil stored air-dry for 5 weeks (see table 31).

Table 31.- The effect of storage on the pounds per acre of available phosphorus in a Dekalb silt loam as measured by the Truog method.

Treatment of soil sample	Length of storage period					
	None	1 week	2 weeks	5 weeks	9 weeks	9 months
Air-dry; stored in laboratory 1/	90	93	98	97	98	121
Air-dry; stored at about 5°C.	90	--	93	--	--	111
Dried 5 hours at 100°C.; stored in laboratory	104	--	111	114	--	138

1/ Samples stored in stoppered bottles tested the same as those stored in open bottles.

As was shown in table 30, storing the soil moist for a few weeks immediately after sampling produced a temporary decrease in available phosphorus. Moreover, alternately moistening and drying the soil was later found to materially increase the solubility of the phosphorus. In order to investigate further the effect of moistening and drying, samples of soil were weighed into extraction flasks and alternately moistened and dried (the soil had been stored air-dry in the laboratory for 9-10 months before this treatment was tried). The results given in table 32 show that keeping the sample moist for 24 hours gave a 33 percent increase in the value for available phosphorus. Neither drying a moist sample nor remoistening a sample that had been moistened and dried had any significant effect on the soluble phosphorus content.

Table 32.- The effect of alternate moistening and drying on the available phosphorus content of a Dekalb silt loam as measured by the Truog method.

Treatment of soil 1/	Available phosphorus (pounds per acre
Control (dry)	123
Moist	164
Moistened and dried	168
Moistened, dried, and moistened	165
Moistened, dried, moistened, and dried	164
Moistened, dried, moistened, dried, and moistened	166
Moistened, dried, moistened, dried, moistened, and dried	180
Moistened, dried, moistened, dried, moistened, dried, and moistened	180

1/ Drying was facilitated by pulling a current of air over the sample. Since all samples were weighed out at the same time and analyzed for available phosphorus 24 hours later, it is obvious that the soil in the second treatment was moist for 24 hours and that the other samples were moist for shorter periods of time.

Samples were then kept moist for different lengths of time before analysis. As shown in table 33, moistening the soil two weeks prior to analysis had about the same effect as moistening 24 hours prior to analysis. Moist samples kept at 5°C. showed the same increase in soluble phosphorus as samples stored at laboratory temperature, indicating that the increase was not the result of microbiological activity. Under other conditions, however, microbiological action apparently may be a factor. Thus a sample of this same soil treated with glucose and nitrate of soda and then alternately moistened and dried several times analyzed only 90 pounds per acre of readily available phosphorus as compared with 162 pounds for the control without glucose and nitrogen.

Table 33.- The effect of moistening on the available phosphorus content of a Dekalb silt loam as measured by the Truog method.

Soil treatment	Available phosphorus (pounds per acre)
Control (dry)	124
Moist 10 hours at about 23°C.	142
Moist 24 hours at about 23°C.	159
Moist 48 hours at about 23°C.	155
Moist 96 hours at about 23°C.	176
Moist 9 days at about 23°C.	151
Moist 14 days at about 23°C.	159
Moist 21 hours at about 23°C.	154
Moist 21 hours at about 5°C.	157

These preliminary studies on soil from an old pasture show that marked changes in phosphorus solubility may occur after the sample is taken from the field. The nature of these changes have not yet been determined.

Pathology

The pathological investigations were begun July 7, 1938, so this part of the annual report covers the period from that time to the end of the calendar year. Very little work has been done heretofore on the diseases of pasture plants, hence there is presented almost a virgin field for research. During the six months the work has been under way at the Laboratory, investigations have been begun in a preliminary disease survey, the isolation of fungi, life history studies, and seed treatments.

Preliminary Disease Survey

The period available for disease survey was rather limited and only included the period from the latter half of July, 1938, to the onset of cold weather. In scope the observations were confined primarily to the experimental plots of the Laboratory and the surrounding territory within a radius of 15 or 20 miles, although one trip was made through southwestern Pennsylvania and West Virginia north of Morgantown in August, 1938.

In the experimental plots the following diseases and organisms were found on the species of grasses and clovers present. Additional observations concerning the territory adjacent to State College, Pa., and southwest Pennsylvania are given where pertinent.

Scolectorichum graminis was found associated with a leaf spot of *Poa compressa*, *Phleum pratense*, *Dactylis glomerata*, *Arrhenatherum elatius* *A. erianthum*, and *Agrostis alba*. The leaf spot caused much more damage in the case of *Poa compressa* and *Phleum pratense* than the other hosts. The disease was difficult to find during the hotter part of the summer but increased with the advent of cooler weather.

A *Septoria* sp. was associated with a relatively severe leaf spot of *Dactylis glomerata*, *Arrhenatherum elatius* and *A. erianthum*. This disease was found only on the plants in the experimental plots and was more severe

on second-year plants than first-year ones.

The rusts attacked many of the species of grasses in the plots, Puccinia coronata being particularly bad on Lolium perenne and Anthoxanthum odoratum. Puccinia graminis phlei-pratensis was rather important on Phleum pratense. No attempt was made to identify other rusts, although collections were made of them.

Some of the other pathogens found associated with diseases of grasses in the experimental plots and surrounding country were:

Colletotrichum graminicola which was rather severe on Agrostis alba and other Agrostis species.

Helminthosporium vagans caused a leaf spot of Poa pratensis.

A Septoria leaf spot of Poa pratensis.

On clovers, particular attention was paid to white clover Trifolium repens

The Cercospora leaf spot of clover caused by Cercospora zebrina was very prevalent during the summer and seemed to cause a rather severe defoliation of second-year white clover plants. The disease was also found on Trifolium pratense, T. dubium, T. hybridum, T. agrarium, and Meliolotus alba. None of the 10,000 second-year white clover plants growing in the experimental plots was completely free of the disease although varying degrees of seeming resistance occurred.

An anthracnose of clover was found in the experimental plots and throughout the southwestern part of Pennsylvania and West Virginia as far south as Morgantown. The fungus has been tentatively identified as Colletotrichum destructivum O'Gara, it differing from C. trifolii Bain and Essary in spore length, width, and shape. It was isolated from Trifolium repens, T. pratense, and T. hybridum. Its importance is unknown.

The sooty blotch of clover caused by Polythrincium trifolii (Cymadothea trifolii), the Pseudoplea leaf spot caused by Pseudoplea trifolii, and the rust of clovers caused by Uromyces spp. were severe in the nursery

on white clover and occurred throughout southwestern Pennsylvania. Clear cut resistance and susceptibility were indicated among the plants in the nursery with respect to Polythrincium trifolii and Uromyces spp. Mosaic of Trifolium repens, T. pratense, and T. incarnatum occurred on a few plants in the nursery.

Other diseases present were Stagnospora leaf spot of white clover, and Macrosporium leaf spot of Trifolium pratense and Medicago sativa.

Isolation of Fungi

Single spore cultures using the Chambers' micro-manipulator and tissue cultures were made as given in table 34. Various other fungi have been isolated, but no definite identifications have been made to date. (Table 34 on page 81).

Preliminary Life History Studies

The time devoted to life history studies has been very limited but some cultural work and a few preliminary inoculations with pure cultures in the greenhouse have been made.

In the case of the fungus tentatively identified as Colletotrichum destructivum, spore measurements were made of 100 spores each from 6 single spore cultures isolated from Trifolium pratense, T. hybridum, and T. repens, and compared with 100 spore measurements each of two cultures of Colletotrichum trifolii obtained from Mr. Lawrence Henson, St. Paul, Minn. The measurements and also the original measurements of the two species as described by Bain and Essary and P. J. O'Gara are given in table 35.

Preliminary inoculation tests in the greenhouse, repeated three times, indicate the cultures used of Colletotrichum destructivum to be more pathogenic to a strain of Melilotus alba than to Trifolium repens, T. pratense, T. hybridum, and Medicago sativa, there being a killing of

Table 34. Isolations and cultures of fungi obtained from legume and grass diseases.

Organism	Hosts	Location	No. cultures	No. collections	Type isolations
Scolecotrichum graminis	*Poa compressa*, *P. nemoralis*, *Dactylis glomerata*, *Phleum pratense*, *Arrhenatherum elatius*, *A. erianthum*, *Agrostis alba*	State College, Pa. Arlington Farms, Va.	87	31	single spore
Cercospora zebrina	*Trifolium repens* (includes Ladino) *T. dubium*, *T. hybridum*, *T. agrarium*, *Melilotus alba.*	State College, Pa. West Newton, Pa.	43	10	single spore
Colletotrichum destructivum	*Trifolium repens* (includes Ladino), *T. pratense*, *T. hybridum*	State College, Pa. West Newton, Pa.	50	23	Single spore
Pseudoplea trifolii	*Trifolium repens* (includes Ladino), *T. hybridum*, *Medicago sativa*, *T. pratense*	State College, Pa.	24	9	Tissue
Macrosporium sarcinaeforme	*Trifolium pratense*	State College, Pa. Washington, Pa.	10	3	Single spore - tissue
Macrosporium sp.	*Medicago sativa*	State College, Pa.	3	1	Single spore
Septoria sp.(a)	*Dactylis glomerata*, *Arrhenatherum elatius*, *A. erianthum*	State College, Pa.	20	7	Single spore
Septoria sp.(b)	*Poa pratense*, wheat	State College, Pa. and environs	3	2	Single spore
Colletotrichum graminicola	*Agrostis alba*	State College, Pa.	3	1	Single spore

Table 35.- Measurements in microns of lengths and widths of 100 spores each of single spore cultures of Colletotrichum destructivum and C. trifolii.

Organism	Host	Length	Width	Measurements in original description
Colletotrichum destructivum	Trifolium hybridum	16.23	3.12	14-22 x 3.5-5mu.
	Trifolium hybridum	16.52	3.57	
	Trifolium repens	17.16	3.71	
	Trifolium repens	17.36	3.64	
	Trifolium repens	17.28	3.85	
	Trifolium pratense	19.22	3.57	
Colletotrichum trifolii	Medicago lupulina	12.06	5.69	11-13 x 3-4mu.
	Melilotus sp.	13.19	5.07	

portions of the petioles and eventual death of some of the plants in the case of this strain of Melilotus alba and only leaf spots and petiole blackening in the case of the other hosts.

Culturally, rather distinct differences in topography, amount of sporulation, type of growth, and color occur among 40 single spore cultures compared on potato dextrose agar. No correlation between cultural characters and host was found.

The cultures when first isolated were much more uniform than after sometime in culture and in several cases lost most of their ability to sporulate. Preliminary studies were then begun to determine if possible the cause of this later variation and partial loss of sporulating ability. As sectors appeared in tubes and Petri dishes, isolations were made from some of them by transfer of hyphae and by single sporing. The sectors gave rise in both cases to distinctly different cultural types which have remained so to date.

Single spore cultures were made also from cultures in which no sectors were visible and compared for variation in cultural characters. A total of 464 single spore cultures were made from 9 original single spore cultures or single spore cultures made in turn from these original 9 isolates and were compared in tubes and plates. Those made from sectors or

from cultures which were blotchy in appearance were different from the original type but isolates from homogeneous cultures were alike.

Spores were fixed and stained using Flemming's weaker solution and iron-alum hematoxylon. Conidia were nearly always uninucleate, only 35 with two nuclei being found among 11,330 spores examined and counted from 8 cultures. These 35 were larger in nearly every case than the remainder of the spores.

Eighty-seven cultures of Scolecotrichum graminis were compared culturally on potato dextrose agar at room temperature (Ca. 70°F.). These cultures fell into 3 primary groups: a white type which included isolates from Poa compressa, P. nemoralis, Phleum pratense, Arrhenatherum elatius, A. erianthum, and Dactylis glomerata; a much more slowly growing, darker rougher type without the white overgrowth which included only cultures from Agrostis alba; a rather heterogeneous group which ranged from dark to light gray and with varying amounts of white overgrowth. This third group included cultures from Dactylis glomerata and two from Arrhenatherum elatius.

It is possible that some of the white group are a result of variation from cultures of the last group, as white sectors isolated from two or three of the cultures of this last group gave rise to cultures of the first group. All cultures from Agrostis alba have been constant and distinct to date. Figure 1, A, gives some of the cultural types.

Various media have been used in an attempt to obtain sporulation of cultures but results have been negative to date.

Preliminary inoculations in the greenhouse with mycelial suspensions from pure cultures have been disappointing so far although some infection has been secured.

Spore measurements of 100 spores each were made from 9 collections of the fungus from 6 hosts and the results are given in table 36.

Table 36.- Lengths and widths of 100 spores each from 9 collections of *Scolecotrichum graminis*.

Host	Place of collection	Av. length in microns	Av. width in microns
Poa compressa	State College, Pa.	38.41	11.12
Poa compressa	Arlington Farms, Va.	39.83	10.54
Poa nemoralis	Beltsville, Md.	40.65	10.35
Phleum pratense	State College, Pa.	34.89	10.41
Phleum pratense	State College, Pa.	38.35	10.46
Dactylis glomerata	State College, Pa.	35.62	9.51
Dactylis glomerata	State College, Pa.	35.73	10.41
Arrhenatherum elatius	State College, Pa.	37.15	11.71
Agrostis alba	State College, Pa.	37.36	10.60

Sectors have been isolated from single spore cultures of *Cercospora zebrina* which differed culturally from the original type but no further study has been made.

Seed Treatment

Preliminary seed treatment studies have been begun with legumes and grasses using 6 fungicides but results have been inconclusive due to lack of sufficient seedling blight and damping off.

SUMMARY

In conformity with the regional plan of coordinating and integrating pasture research in the Northeastern States, two important meetings were held during the year. In March, plant breeders, meeting in New York City, discussed problems of mutual interest and developed general plans for a coordinated attack on pasture improvement by breeding. In September, the collaborators held their second annual meeting at State College to review the research under way at the Laboratory. In addition to these meetings a closer relation among pasture research men in the Region was fostered by bringing up to date and exchanging project outlines and by encouraging an exchange of professional views through personal contact and correspondence.

A number of pasture research men working at the State stations have visited the Laboratory and several members of the Laboratory staff have visited State stations.

During 1938 facilities for research in plant pathology were established at the Laboratory. A pathologist was added to the staff, the pathology laboratory was equipped, and a new greenhouse, 122 feet by 35 feet was constructed.

Inasmuch as a partial summary of the research activity of the Laboratory may be found at the end of each pertinent section, only a very brief summary will be attempted here. The research of the Laboratory is organized around (a) cytogenetics and breeding, (b) physiology and chemical composition, and (c) pathology.

(a) Progeny rows from selected plants of Kentucky and Canada bluegrass have been established as the first step in a study of the method of reproduction in these species. A high incidence of twinning was found among the progeny of certain Kentucky bluegrass plants and in some cases the two seedlings constituting a pair of twins exhibited marked morphological differences. Parchment bags for the grasses and finely woven cloth bags for the legumes have proved satisfactory as isolating agents in obtaining selfed seed. Some manipulation of the flowers seems necessary to obtain a good seed set with white clover. Hot water for emasculating grasses and suction for depollinating white clover have given encouraging results. A wide variation in self fertility as determined by seed set under bag has been found in perennial ryegrass, orchard grass, timothy, and white clover. On the average, first-generation inbred progenies of white clover were about 70 percent as vigorous as the parents. _Lolium perenne_ has been successfully hybridized with _Festuca elatior_. Self sterile perennial ryegrass plants showed highly variable cross fertility. White clover was crossed readily with

Ladino, but the interspecific crosses attempted within the genus <u>Trifolium</u> were unsuccessful. A number of differential characters of possible value for genetic studies have been noted in white clover, ryegrass, and orchard grass. Preliminary evidence suggests tetrasomic ratios in orchard grass. and that HCN content of white clover is heritable. The alkaloid colchicine has proved effective in inducing chromosome reduplication in perennial ryegrass, red, clover, and white clover. Something over 1200 sod plots have been established by asexual propagation from various pasture grasses and white clover as a first step in attempting to evaluate plant types for pasture purposes.

(b) Kentucky bluegrass, orchard grass, and timothy apparently are stimulated to head by low temperatures, followed by relatively long days (artificial light). Canada bluegrass, on the other hand, requires only the long day to induce heading. Preliminary vernalization experiments suggest that Canada bluegrass and perennial ryegrass may be forced into heading from 30 to 35 days after planting. A device for automatically irrigating pot cultures with nutrient solutions has been developed and found satisfactory. Greenhouse experiments show that clones of white clover respond differently to a given level of soil fertility. In chemical tests made thus far about one-fourth of all white clover plants have been found to contain variable amounts of hydrocyanic acid. No toxicity to animals from this substance, even with the plants of highest concentration, has as yet been demonstrated. A considerable variation in total nitrogen has been found in the leaves and culms among individual plants of Kentucky bluegrass. Some improvement in chemical methods for the determination of carbohydrates in grasses has been made. The subjection of moistened seeds to relatively low temperatures proved effective in inducing germination of freshly harvested Kentucky bluegrass seed. In studies of asexual propagation of Kentucky and Canada bluegrass, clonal pieces rooted readily but

auxilin was found to be of questionable value in stimulating better rooting. A high level of nutrients in the medium in which the clonal pieces were inserted, rather than the size of the original clonal piece, was important in conditioning the subsequent growth. Transplanting clonal pieces directly from the mother plants in the greenhouse to the field proved satisfactory with the grasses but not with white clover. Preliminary studies show that marked changes may occur in the soluble phosphorus of pasture soils after the soil samples are collected.

(c) Pathological investigations with pasture species have been initiated to determine the most important diseases now occurring in pastures of the Region, to work out life histories of certain pathogens, and to ascertain the effect of seed treatment in controlling damping off.

LIST OF PUBLICATIONS AND PAPERS PRESENTED BEFORE NATIONAL SOCIETIS

Atwood, S. S. A "One-Leafed" White Clover. In the Jour. of Hered. Vol. 29, No. 6, pp. 239-240, 1938.

Garber, R. J. United States Regional Pasture Research Laboratory. In the Herbage Reviews. Vol. 6, No. 3, pp. 146-150. 1938.

*Atwood, S. S. Self and Cross Sterility and Fertility.

*Myers, W. M. Inbreeding and the Utilization of Inbred Lines.

*Sprague, V. G. The Physiological Approach to Pasture Problems.

**Sullivan, J. T. Report on Carbohydrates in Plants.

* Paper presented before the American Society of Agronomy at the annual meeting held in Washington, D. C., November 16-18, 1938.

** Paper presented before the Association of Official Agricultural Chemists at the annual meeting held in Washington, D. C., November 14-17, 1938.

Appendix A

PROGRESS REPORTS OF PASTURE RESEARCH AT STATE STATIONS

Appendix A contains progress reports of pasture research under way at the twelve northeastern State agricultural experiment stations. In some cases these reports cover the period for the last fiscal year, beginning July 1, 1937, and in some cases for the calendar year 1938. In most instances they are reproduced as submitted by the collaborators, but it was necessary to abbreviate somewhat a few of the reports so as not to make the Annual Report as a whole too bulky. It is hoped that these abridgments have not seriously impaired the value of the progress reports, since they make possible within a single comprehensive report a survey of the status of pasture research in the northeastern United States.

The data and descriptions contained in Appendix A belong to the cooperating State stations and are <u>not released for publication</u>.

Connecticut (Storrs)

<u>The Effects of Fertilizer Treatments on the Soil, the Flora, and the Production as Measured by Grazing</u>

In order to present a better picture of the effects of the surface applied fertilizers than afforded either by the results of one year or one period, the average annual productions of the several pastures by three- or five-year periods are presented on page 89 in tabular form.

Explanation of symbols:

P - superphosphate (16 percent P_2O_5) at 500 pounds per acre at beginning of each period.

K - muriate of potash (50 percent K_2O) at 100 pounds per acre at beginning of each period.

L - limestone at 1 ton per acre in 1924 and 1929, excepting for numbers 10 and 11, where limestone at 2 tons per acre was applied in 1932.

N - nitrogen at 28 pounds per acre at each application.

NN - nitrogen at 56 pounds per acre at each application.

Number	Fertilization	Feed units (therms) per acre, annually, as measured by grazing, 1921-1937				
		First 1/ period 1921-23	Second period 1924-28	Third period 1929-31	Fourth period 1932-34	Fifth period 1935-37
1	No treatment	711	675	544	543	542
2	P	726	1126	1040	870	973
3	PL	615	1237	1449	1202	1142
4	PLK	723	1451	1517	1148	1201
5	PK	643	1083	1040	945	999
6	LK	735	793	750	637	791
7	PKN1	700	1576	1688	1417	1448
8	PLKNN1	733	----	1856	1688	1591
9	PLKN12	733	----	1743	1833	1628
10	PLKN123	637	----	----	1657	1847
11	PLKN23	637	----	----	1502	1655
12	PKN2	700	----	----	1240	1213
13	PKN3	643	----	----	1140	1103

1/ During this period no fertilizers were added to any of the pastures.

The numbers after the letters N or NN, refer to time of applying the nitrogen: 1 means April; 2, June; and 3, August applications. Thus, N123 means that nitrogen at 28 pounds per acre was added in April and repeated in June and August of each year.

These productions of pasturage by periods are remarkably consistent for each of the several systems of fertilization. It is evident, however, in the sases of those treatments for which data are available throughout the fourteen years since the first application of fertilizers, that the productions of the last six years were somewhat lower than for the previous eight years. Several factors may have had a part in bringing about this result:

1. Dairy heifers instead of beef steers grazed the plots during the last six years.

2. White clover has been much less prevalent in recent seasons. This has been true especially of the "no nitrogen" pastures. Nevertheless,

those receiving nitrogen and consequently less dependent on clover for their supplies of nitrogen also declined in yields.

3. Unfavorable weather, particularly less precipitation, would have an important influence on production, but in this case nearly every month from April through October averaged more during the last six seasons (the period with lower yields), than during the earlier eight-year period.

Although it has had as much lime and potash as any in this experiment, the plot which has received no phosphorus since 1924, when superphosphate (16 percent P_2O_5) at 500 pounds per acre was applied, is now showing distinct vegetative symptoms of deterioration. For example, an adjacent plot, phosphated at the beginning of every period, has eight times as much clover, nearly twice as much bluegrass, but only two-thirds as many weeds and bare spaces. In this case, deterioration of the pasture, due to insufficient phosphorus, was apparent in the chemical analyses of the herbage and soil several years before it was evident in the botanical composition.

Limiting lime to the single ton of limestone applied in 1924 has had little effect on the character of the vegetation or on the production of pasturage.

For the first time since the first limestone was applied, the surface inch of soil from the plots which received limestone at one ton per acre in 1924 and again in 1929, is more acid than the second or third inches. With the passage of time, there has been a gradual reduction in the differences in pH between the several one-inch layers of soil from the limed plots. In other words, the bases in the soil, chiefly lime, are being carried slowly downward by natural forces such as rain water. The mixing of the soil by earth worms and the decomposition of grass roots probably have had slight, but similar, effects. These statements are illustrated by the following data, which summarize the results of testing the soil from seven limed and seven unlimed plots:

Depth (inches)	Increases in pH (decreases in acidity) due to limestone applied at one ton per acre in 1924 and 1929 on the surface of untilled pastures		
	1933	1935	1938
0-1	0.61	0.68	0.42
1-2	0.43	0.53	0.46
2-3	0.22	0.31	0.42
3-4	0.17	0.21	0.32
4-5	----	0.12	0.27
5-6	----	----	0.18

A conclusion of practical value derived from these studies is that moderate amounts of limestone applied on the surface of grassland affect the soil, in the range of most of the grass roots, more and for a longer period than if mixed with the plowed layer.

The Effects of Various Chemicals on the Soil, on the Botanical Composition of the Sward, and on the Stands and Growth of Kentucky Bluegrass and Rhode Island Bent Grass

The title suggests the broad objects of this project. It suggests, also, that considerable time should elapse before drawing conclusions or terminating the experiment.

The past year was the second season during which the scheduled chemical treatments have been applied to these plots of grass. The Kentucky bluegrass plots were cut with a lawnmower seven times and the Rhode Island bent grass plots six times in 1937 to determine the yields of dry matter. Samples from each cutting and treatment have been analyzed for nitrogen and later it is planned to make determinations of the calcium, magnesium, sodium, and potassium content of the grasses. Soil samples by one-inch layers were taken of the upper three inches of each plot in November.

Based on the averages for two years, the plots receiving from different sources 29 pounds of nitrogen per acre in April, June, and August

(84 pounds per year) varied in yields of dry matter per acre from 1025 to 2016 with Kentucky bluegrass and from 1543 to 3098 with Rhode Island bent grass. On this soil and original pH of 515, ammonium carbonate and ammonium chloride have been consistently poor sources of nitrogen, while calcium nitrate and calnitro have been somewhat superior to the others. This is the second experiment in which Rhode Island bent grass has produced more than Kentucky bluegrass under each of a large number of different treatments. This result is contrary to our opinion of a few years ago.

Adding sodium chloride, sodium sulphate, sodium carbonate or potassium carbonate to the plots receiving urea has had no marked effects on the growth of either grass.

Doubling the application of calnitro (168 pounds of nitrogen per year) increased the yields of Kentucky bluegrass 74 percent; of Rhode Island bent grass only 37 percent.

When the same total amounts of nitrogen were used, monthly was not superior to bi-monthly fertilization. However, a somewhat more uniform growth and nitrogen content were maintained by the smaller but more frequest treatments.

As in another experiment, the peak of production has been changed from the June norm to either July or August by withholding April nitrogen and applying 56 pounds later in the season.

In the case of Rhode Island bent grass, the average nitrogen content of the herbage varied in 1937 from 2172 percent for the ammonium carbonate treatment to 2.98 percent for urea plus sodium carbonate. The double amount of calnitro increased the average percent of nitrogen from 3.02 to 3.50. With Kentucky bluegrass the corresponding figures are practically the same. The recoveries of nitrogen from the various treatments have not been calculated at this time.

At the end of the second season in this experiment no appreciable differences existed in the botanical compositions of the variously treated plots. However, the deneness of the sward corresponds to the yields. In time it is probable the advent of weeds and clover will depend to a considerable extent on the denseness of the grass.

Very marked differences were found in the reactions of the soil sampled in November 1937. The surface inch showed the greatest changes due to the chemicals. For example, in two years sulphate of ammonia has made the surface inch of soil 0.88 pH more acid, while cyanamid has caused almost an equal fluctuation toward alkalinity or a total difference of 1.73 pH. In rapidly decreasing degrees, the effects of these fertilizers extended to the third inch of soil.

Sodium nitrate has influenced the reaction of the surface inch less than cyanamid or sulphate of ammonia but the second and third inches much more than any of the carriers of nitrogen. Evidently this result is due to the mobility of the sodium ion, for where sodium chloride or sodium sulphate were applied, the first inch was consistently more acid and the second and third inches less acid, indicating that the acid radical was absorbed by the surface soil while the alkaline sodium ion migrated further downward.

The differential effects on the soil of two very similar compounds is illustrated below:

Compounds added with urea to surface of the soil	Total pounds per acre 1/	Changes in pH of soil: 0-1 inches	1-2 inches	2-3 inches
Sodium carbonate	660	+0.68	+0.56	+ 0.37
Potassium carbonate	330	+0.08	-0.03	+ 0.07
None (urea only)	---	-0.05	+0.04	- 0.04

1/ When used in these amounts, 216 times as many sodium as potassium ions were applied.

Probably much more of the potassium was absorbed by the grasses. Also more potassium may have been "fixed" in a relatively inactive state in the soil. Further analyses of both grass and soil must be made to obtain definite explanations for these results.

Other Projects

The application during six years of a total of 1800 pounds of sulphate of ammonia and 600 pounds of nitrate of soda per acre to the surface of Kentucky bluegrass plots has increased the acidity of a soil, with an original pH of 6.0, to such a degree that the grass has become very thin and unhealthy in appearance and sorrel is rapidly occupying the space vacated by the grass. To a much lesser extent, this is also true of Rhode Island bent grass. In both cases, the plots with the best appearance two years ago are now among the poorest. It is concluded from these results that one should use lime in conjunction with sulphate of ammonia or substitute a source of nitrogen which does not increase so rapidly the acidity of the soil.

Although most of the acidifying effects of the sulphate of ammonia were in the surface two inches of soil, the fourth inch was slightly more acid than the corresponding layer in the undertilized plots.

The propensity of Kentucky bluegrass for soils rich in lime is illustrated by some botanical analyses of some permanent grass plots limed at widely different rates in 1930

Tons of limestone per acre	Percent of area occupied by Kentucky bluegrass in May 1938 (W2)			
	Limestone only	Limestone plus phosphorus	Limestone plus phosphorus plus potash	Average
2	trace	4	4	3
4	6	2	4	4
8	8	20	12	13
16	18	40	28	29

Because there were lesser amounts of bluegrass where potash was used, it does not follow that it can thrive on low levels of soluble potassium. Clover has spread so rapidly after adding potash that it offers strong competition for other species.

The time of application of nitrogenous fertilizers is important in their influence on the prevalence and growth of species. Thus, a fall treatment with sulphate of ammonia had little effect; a spring application doubled the area occupied by red fescue, but reduced the white clover over 80 percent. Similar differences were observed in the grass and clover population when nitrate of soda was added all in April, rather than divided between April and June.

The common grasses, timothy, orchard, Kentucky bluegrass, and Rhode Island bent, are much less sensitive to deficiencies in calcium, phosphorus, and potassium than are the legumes, alfalfa, sweetclover, and red and white clovers.

The Adaptability of Varieties and Species of Grasses and Clovers for Pastures

During the first season (1936) after being sown in March on the surface of land previously seeded with nine different grasses in pure culture, Ladino clover increased the total yields much more than did similarly planted "Kent" wild white clover or the applications of fertilizer nitrogen equivalent to over 500 pounds per acre of nitrate of soda. Similar results were obtained in the second season (1937). Not only did Ladino produce the largest total yield of dry matter, but led in all periodsexcepting the first half of May.

In another experiment where eighty-three different seed mixtures are under test, those which include Ladino clover have out-yielded all others by wide margins. The ability of Ladino to maintain good stands for several years whether cut for hay or pastured, its tolerance to a wide variety of

soil conditions, and its large production of nutritious forage lead us to believe this legume should appear in every clover-grass mixture seeded in this region.

If Ladino clover could be introduced into our untillable or difficultly tillable permanent pastures, their total, and especially their midsummer, productions would be increased. Attempts to do this have so far proved rather unsuccessful, excepting on recently cut-over lands, where little vegetation existed at the time of seeding. Nevertheless, further trials are contemplated.

When harvested for two years with a lawnmower under conditions simulating controlled lenient grazing, tall oat, meadow fescue, smooth bromegrass, and perennial ryegrass were reduced to less than 20 percent stands and common strains of orchard grass and timothy to less than 50 percent. Of nine timothy strains, only the extreme pasture type, S50, developed at the Welsh Plant Breeding Station, had over 40 percent stands at the end of two years. The stands and yields of the S50 strain, however, were lower than Kentucky bluegrass. It is planned to repeat the timothy strain tests under more lenient systems of harvesting.

At the end of the second season, English and New Zealand strains of perennial ryegrass had 60 and 70 percent stands, respectively, but their yields were also lower than Kentucky bluegrass. All strains of perennial ryegrass tested so far have produced very little palatable forage in June, July, and August. This species thrives in regions with cooler, more uniform temperatures, such as prevail in Great Britain, where it is the dominant grass in permanent pastures.

It is becoming increasingly evident that it will be a long tedious task to find or develop species or strains of grasses and legumes which will supplant the ones commonly found in the northeastern United States.

Relation Between Physical and Chemical Characteristics of the Soil and the Response to Fertilization of Vegetation on Permanent Pastures

During the past year, no new data were secured on this project. However, some work was done to test the practicability of using the centrifuge to determine physical differences in soils, particularly their retention of moisture when subjected to centrifugal force.

Causes of Fluctuations in the Prevalence of White Clover

White clover is by far the most important legume for permanent grassland in northeastern United States. Without liberal use of nitrogenous fertilizers, cloverless grasslands usually produce low yields. Even on soils where white clover thrives under favorable conditions, there are great fluctuations from year to year in the prevalence of clover on the same field. The reason for these fluctuations are obscure. At present out studies on this problem include: (1) strains and varieties of seed; (2) fertilization, including minor elements; and (3) management. The results to date point to the following conclusions:

1. Variety, strain, or source of seed is a very important factor in the longevity of white clover. Under normal lawn conditions, the so-called "wild" or old permanent pasture types have maintained better stands than Dutch clover from Poland or from several parts of the United States.

2. White clover is more sensitive than most grasses to soil deficiencies in such major fertilizer elements as phosphorus, potassium, and calcium. Under a wide variety of managorial conditions, from closely grazed pastures to mowed meadows, phenominal increases in clover without seeding have followed the addition of phosphorus or potassium, where either was deficient. Although white clover has had consistently good stands in grassland receiving stable manure, the more commonly deficient minor element

such as magnesium, boron, manganese, and copper, in addition to calcium, phosphorus, and potassium, have been used with apparent effect. In practically all cases, nitrogen, especially from the physiologically acid carriers, has reduced the proportion of clover in grass-clover stands. Peat moss or grass clippings seem to favor the spread of white clover.

3. Any factor which makes for a more solid stand of grasses tends to reduce white clover. This is tantamount to saying that our native grasses are stronger competitors for space than is white clover. For example, under six different seeding and fertilizer treatments, the permanent pasture and lawn grasses - Kentucky bluegrass and Rhode Island bent grass - had 18 percent of the area in clover, while plots of orchard grass and timothy,with much less solid stands, averaged 42 percent clover. Still poorer stands of several other grasses had 80 percent clover. Another example is furnished by the decrease of white clover in a grazed permanent pasture from 75 percent in the second year, after an essential but deficient plant nutrient had been applied, to 14 percent five years later. During the five years, the increase in grasses and especially Kentucky bluegrass was approximately equal to the decrease in clover.

These statements indicate the complexity of the problem and that probably several factors are active in most cases. Apparently there are many different strains of the native or "wild" white clover and each may respond differently to a given set of conditions. Thus, very close mowing favors the extremely low growing types of white clover.

Effect of Subsoil on Response of Pasture Species to Fertilization

No additional data were obtained during the past year.

Publications, 1937-38

Brown, B. A. The effects of Fertilizers on the Soil, Botanical and

Chemical Composition of the Herbage and the Seasonal and Total Production of Grassland in Connecticut. Report of the 4th International Grassland Congress, pp. 313-317, Aberystwyth, Great Britain. 1937.

Brown, B. A. Fertilizers for Potatoes (third report). Storrs Agr. Expt. Sta. Bul. 223. April 1938.

Delaware

This project deals with the selection and improvement of Korean Lespedeza by pure line methods for Delaware conditions. Originally 3000 selections were made in the greenhouse and later transplanted to the field. Three hundred of the more desirable selections were retained in 1935 for further observation. Three years' seed yield and two years' forage yield were obtained on 300 selections. One hundred of the more disease-resistant strains have been retained for further observation. These were on test at Newark this past season in rows and broadcast plots and in broadcast plots in southern Delaware.

Maine

Permanent Pasture Studies

Fertilizer tests on five permanent pastures were started in 1935. The pastures were located near the towns of Oakland, Farmington, Newport, Anson, and Auburn, Maine. The soil type is distinctly different for each locality. A soil survey has not been made of these areas and nomenclature for the soils worked with cannot be given.

The Anson pasture is located on crop land which had received some farm manure in the past. It was very unproductive under no fertilizer treatment. The Newport pasture had never been fertilized or limed, but was fairly productive without added fertilizer. The Oakland, Farmington, and Auburn pastures were typical unproductive, "run-out" permanent pastures that

had never been treated.

The fertilizer tests on each farm originally consisted of twelve different treatments in duplicate, each test plot being one square rod in size. Later each test plot was subdivided into thirds to study the effect on yield of repeating the original treatment or, in certain cases, changing the treatment to secure other information.

The herbage was cut with a lawn mower at least twice during early spring, once during midsummer, and once in the fall, except for the 1937 season when a fall cutting was not available due to a prolonged drought. Immediately after harvesting each plot green weight was determined and a 500-gram composite sample saved which was shortly oven dried for moisture determination.

The Effect on Yield of Applied Fertility Elements

The effect of surface applications of the major plant food elements and lime to the pastures studied is shown in tabular form on page 101. A need for the three major plant food elements as the initial step in permanent pasture improvement is clearly indicated.

Phosphorus alone, on the basis of yearly comparisons (average of five farms) did not give a significant increase in yield over no treatment; however, over the three-year period the odds indicate that the average annual increase of 10.0 percent was significant. Phosphorus alone was also used at a rate of 120 pounds of P_2O_5 per acre but proved very little more effective than the 60-pound rate. Phosphorus and lime proved somewhat more effective, giving a three-year average annual increase of 17.7 percent over no treatment. The addition of potash to phosphorus and lime brought about a very desirable change in the amount and vigor of native white clover and this was reflected in a very significant three-year average annual increase of 48.6 percent over no treatment.

Year	Treatment in pounds				Yield in pounds absolute dry matter (average of duplicate plots)					Average yield
	$N\text{-}NO_3$	P_2O_5	K_2O	Lime	Oak.	Farm.	New.	Anson	Auburn	
1935					584	1460	2112	744	1480	1276
1936	(no treatment)				672	1248	1984	1096	1112	1222
1937					736	992	1664	960	736	1018
							Three-year average			1172
1935		60			664	1624	2016	1256	1528	1418
1936	(Phosphorus only)				784	1319	1856	1896	1144	1400
1937					528	1056	1488	1488	744	1061
							Three-year average			1293
1935		60		2000	840	1456	1920	1264	1496	1395
1936	(Phosphorus; lime)				848	1344	2192	2072	1184	1528
1937					768	1232	1504	1632	928	1213
							Three-year average			1379
1935		60	60	2000	848	2200	2920	1168	1792	1786
1936	(Phosphorus;potash;lime)				1168	1992	2720	2168	1424	1894
1937					864	1680	2400	1824	960	1546
							Three-year average			1742
1935	60	60	60	2000	2112	2589	3376	1858	2480	2481
1936	60 (Phosphorus;potash; lime; nitrogen annually)				1952	2784	3416	2856	2176	2637
1937	60				1392	2472	3096	2904	1896	2352
							Three-year average			2490
1935	60	60	60		1888	2968	3096	1720	2456	2426
1936	60 (Phosphorus;potash; nitrogen annually)				1800	2872	3088	2576	1992	2466
1937	60				888	2424	2616	2904	1632	2093
							Three-year average			2328

It is to be noted, from the individual farm yields, that a marked response from phosphorus alone, and phosphorus plus lime, was obtained only on the Anson pasture. Further, the addition of potash to phosphorus and lime on this pasture had little effect on yield except during the 1937 season. This is accounted for by the fact that this pasture is located on crop land and previous treatment with farm manure had built up an appreciable reserve of available potash. Without added potash this reserve was showing signs of depletion by the close of the 1937 season.

The effect of nitrogen in conjunction with phosphorus, potash, and lime

was to more than double the yield over no treatment, giving an average annual increase for the three years of 112.5 percent. Nitrogen, phosphorus, and potash without lime over this same period gave an average annual increase of 98.6 percent.

Top dressing with limestone did not prove particularly beneficial over the short period of these tests. The writer has observed that pastures adequately fertilized without the use of limestone become less acid and well supplied with available calcium in the course of time. Apparently, calcium brought to the surface by roots, and there released upon the decomposition of the roots, plus the continuous return of calcium by way of feces and urine, together with the appreciable quantities of calcium added as calcium sulphate when applying superphosphate, are sufficient to maintain and even build up the calcium supply of the surface soil.

Effect of Additional Minerals Without Nitrogen on Dry Matter Yields

The data show the need for rather frequent applications of the minerals phosphorus and potash when used without nitrogen during the early stages of pasture improvement. The yield on each farm under no additional minerals was falling by the close of the 1937 season but was restored or improved upon by repeating the initial application. The increases in yield were due entirely to the more vigorous growth of white clover. Calculated odds in favor of the spring over the fall applications of minerals are only 1:1 and not being significant indicate that the minerals may be applied either in early fall or early spring with equal effectiveness.

Effect of Additional Minerals with Nitrogen on Dry Matter Yields

The yield on the third of the plot receiving only nitrogen for 1937 fell off markedly on each pasture except at Anson, but in all cases was restored

or very definitely improved upon by additional minerals.

The data further indicate that, except for the Oakland pasture, which was very unproductive to start with, there was little to be gained from a second application of minerals appreciably larger than 60 pounds per acre each of phosphoric acid and potash.

Effect of Small Annual Versus Larger Initial Applications of Minerals on Dry Matter Yields

The effect of spreading an initial application of phosphorus and potash (60 pounds phosphoric acid and 60 pounds potash per acre) over a three-year period at twenty pounds each of phosphoric acid and potash annually is shown by the data collected in 1935, 1936, and 1937. For the three-year period on the five pastures, grand average, there is no significant difference in favor of either method. As would be expected, the larger initial application of minerals was most effective the first year and somewhat less effective each succeeding year. The fact that the average annual yield over the three-year period is practically the same for both methods of application indicates that in the early stages of pasture improvement the vegetation makes, percentably, almost quantitative use of applied minerals with nitrogen.

Effect on Yield of Other Fertilizer Treatments

The effects of yield of several other fertilizer treatments were studied but no significant increases or decreases were obtained. Deriving a sixty-pound nitrogen application from several sources of nitrogen, i.e., ammonium sulphate, urea, and sodium nitrate, proved to be of no advantage over a straight sodium nitrate treatment.

Applications of magnesium, manganese, boron, and iron gave no significant increases. An explanation for a lack of response from the minor plant foods is that these pastures respond so markedly to additions of the

major plant foods that normal variations in these large increases apparently offset the beneficial effects of the applied minor plant foods. Further studies along this line will be made on the pasture paddocks at Highmoor Farm as soon as these paddocks no longer give marked response to additions of phosphorus, potash, and lime.

Effect of Fertilization on Chemical Characteristics of the Soil

Data were collected to show the effect of rather liberal complete fertilization on the chemical characteristics of the soils considered. It is to be noted that desirable increases in available plant food did not occur beyond the surface 0-1-inch layer. The treatment given, however, greatly improved the fertility of the surface inch.

These data further show the advisability of selecting the most fertile acres available for permanent pasture improvement. Soil in which the sub-layers are already in a reasonably fertile condition (reasonably fertile crop land for example) will offer a much deeper feeding zone for permanent grasses. In all probability it will take many years of treatment when starting with an impoverished permanent pasture soil to appreciably raise the level of soil fertility by surface applications beyond the surface inch.

It was previously mentioned that the Anson pasture did not initially respond to added potash. The data show that the soil of this pasture carried about 62.3 p.p.m. of available potash throughout the sub-layers (equivalent to 125 pounds of available potash per acre, six and two-thirds inches). This amount of available potash is sufficient for excellent pasture growth as indicated by yield data.

The data clearly indicate the desirability, if not the necessity, of obtaining soil samples in one-inch layers when studying changes in chemical characteristics of permanent pasture soils under diffierential fertilizer treatment. It also raises the question of the proper method of obtaining

soil samples from farm pastures from which recommendations for improvement are to be given on the basis of soil analysis.

Many additional chemical studies are under way to determine the adequate level of soil fertility, with respect to all plant food elements, for the production of excellent permanent pasture. These studies will be reported later.

Pasture Experiments on Highmoor Farm

The first section of the tabular matter on page 106 shows the treatment given five permanent pasture paddocks at Highmoor Farm. The second section shows the yield obtained for treatment indicated, together with cost of treatment and value of increased milk production at an assumed price of $2.00 per cwt. of milk.

These paddocks were originally a part of an old "run-out" permanent pasture that had never been plowed, limed, or fertilized. The treatments given paddocks 6, 7, 9, and 10 will be repeated each year until no further increase in milk yield per acre is observed. When maximum milk yield is obtained for any given paddock an attempt will be made to determine the annual fertilizer treatment necessary to maintain excellent production. Paddock 8, no treatment, is maintained chiefly for observational and demonstrational purposes and will remain untreated.

The importance of liberal complete fertilization during the early stages of permanent pasture improvement is clearly shown. A true picture of cost relations cannot be gained until more time has elapsed. Obviously the costs of liberal initial fertilizer treatments should be spread over a period of years. These paddocks, together with several others constructed during the past year, will be reported upon in more detail as more information is available.

Treatment	Cost for pasture season	
	1936	1937
Paddock 6 - Minerals only		
August 25, 1935 - 1 ton limestone	$ 7.50	
April 25, 1936 - 450 lbs. 0-20-20	8.11	
	$15.61	
April 28, 1937 - 400 lbs. 0-20-20		$ 7.60
Paddock 7 - Complete		
August 26, 1935 - 1 ton limestone	$ 7.50	
April 25, 1936 - 1000 lbs. 10-9-9	18.10	
	$25.60	
April 26, 1937 - 1000 lbs. 6-8-8		$13.60
Paddock 8 - no treatment	- - -	- - -
Paddock 9 - Manure-phosphate, top dressed		
August 26, 1935 - 1 ton limestone	$ 7.50	
November 16, 1935 - 15 ton manure	30.00	
November 16, 1935 - 300 lbs. 40 percent superphosphate	6.00	
	$43.50	
October 5, 1936 - 10 ton manure		$20.00
October 5, 1936 - 100 lbs. 40 percent superphosphate		1.90
		$21.90
Paddock 10 - Manure-phosphate, plowed and seeded		
This paddock received exactly the same treatment as paddock 9 except that it was plowed and seeded to timothy, Kentucky bluegrass, and white clover in the fall of 1935	$43.50	$21.90

	Paddock 6		Paddock 7		Paddock 8		Paddock 9		Paddock 10	
	1936	1937	1936	1937	1936	1937	1936	1937	1936	1937
1/Yield-cow pasture days	56	75	104	105	28	27	60	72	120	135
-milk, pounds	1400	1850	2600	2625	700	675	1500	1800	3000	3375
Increased yield of milk over paddock 8	700	1175	1900	1950			800	1125	2300	2700
Value of increased yield of milk over Paddock 8 at $2.00 per cwt. (dollars)	14.00	23.50	38.00	39.00			16.00	22.50	46.00	54.00
Cost of treatment (see foregoing tabulation)	15.60	7.60	25.60	13.60			43.50	21.90	43.50	21.90

1/ Yield figures on standard cow basis, or a 1000-pound cow giving 25 pounds of 4 percent milk and obtaining all feed from paddock stated. Total digestible nutrient yield method of Knott, Hodgson, and Ellington, Washington Agricultural Experiment Station, Bulletin 295, used to calculate yield figures.

Summary and Conclusions

Complete fertilization is essential for the best increases in herbage during the early stages of pasture improvement.

On "run-out" Maine permanent pastures the amount of available potash is equally as deficient as the amount of available phosphorus

During the early stages of pasture improvement minerals (P and K) with nitrogen may be added in rather large infrequent, or smaller frequent, applications with about equal effectiveness. The minerals are equally effective when applied in early fall or in spring. The important factor, for maximum increases, is to "build up" the pasture soil in available minerals as quickly as is warranted.

Nitrogen, with phosphorus and potash, gave the largest increases in yield. Significant differences in yield were not obtained in favor of a sixty-pound nitrogen application per acre derived from ammonium sulphate, urea, and sodium nitrate (twenty pounds nitrogen from each) over a straight sodium nitrate application of sixty pounds of nitrogen.

A significant increase in yield from applications of magnesium, manganese, boron, or iron, was not obtained.

Surface applications of fertilizers did not improve the level of soil fertility beyond the surface 0 to 1 inch layer during the period of the test.

At Highmoor Farm a series of grazing experiments were started in the fall of 1935. The importance of liberal complete fertilization as the initial step in permanent pasture improvement is clearly shown.

Maryland

The Effect of Fertilizer on Fertility and Grass Population of Pastures

This is a study to determine the effect of present pasture manage-

ment practices on pasture fertility. It is concerned with the improvement in quality of pasture turf by the use of commercial fertilizer. The effect of the different fertilizers upon the grass population was also noted. After four years, the results indicate that a fall application of fertilizer is more effective than a spring application. The use of a fertilizer quite high in phosphoric acid and carrying a small amount of potash seems to be the most effective in maintaining a high state of fertility. The use of manure along with the phosphoric acid and potash also stimulated the growth of grass. The movement of phosphoric acid downward into the soil is very slow. The fall application of the fertilizer seems to have favored the penetration of the phosphorus. The comparative response of Lespedeza striata and Lespedeza stipulacea to high acidity has been quite common in some of our test plots. The common Japan clover seems to thrive on soil that is much too acid for the Korean strain.

Improvement of Permanent Pastures in the Several Soil Provinces of Maryland

Objectives: (1) to determine if strains of permanent pasture plants can be selected which are better adapted to conditions in the different parts of Maryland than existing commercial stocks; (2) to learn the conditions necessary for the most economical yield of these strains; (3) to propagate and put into commercial use any superior strains which may be obtained. Local strains of Kentucky bluegrass, orchard grass, perennial ryegrass, and white clover have been isolated, which differ markedly in yield, response under close clipping, and chemical composition. Some of these differences are very distinctly inherited through the seed of bluegrass and to a less extent in perennial ryegrass and white clover. Seed stocks of promising strains are being multiplied and tested. Studies are in progress on the effect of different degrees of closeness of clipping of

ryegrass and different strains of bluegrass upon growth, production, and root development.

Massachusetts

Pasture Breeding

Miscellaneous grasses and legumes- Several hundred strains of grass and legume species were seeded in single rows and in broadcast plots this past August. These seeds were collected from both foreing and domestic sources and included strains of timothy, orchard grass, ryegrass, meadow fescue, white clover, red clover, alsike, and other miscellaneous species. The object of the experiment is to study as many different strain types as possible in order to get some ideas about what types to select for in a future breeding program.

Ladino Clover

The space planted Ladino clover nursery set out in 1937 produced a wide variation in morphological plant types. Some 224 selections were made which included representatives from each rather definite morphological type in order to study each type more closely. The object here, as in the above experiment, is to learn, if possible, what types should be looked for in a breeding program.

Permanent Pastures

Results from outlying fertilizer top-dressing experiments in Worcester County, Massachusetts, together with numerous observations on other permanent pastures throughout the State, lead to the conclusion that at best fertilizer top dressings on old pasture sods will bring these sods up to a moderate level of productivity. Even at this productivity level,

herbage production is seasonable. Herbage yields reach peaks in early summer and early fall and drop to low levels in mid and late summer.

The first limiting factor to the presence of white clover in old pasture sods is moisture. Native white clover can be found widely distributed after wet seasons, but after dry seasons it will be found only in areas where good moisture relationships have been maintained throughout most of the growing season. Lack of available mineral elements, also a limiting factor to the prevalence of white clover, is, from the standpoint of yield, as important as favorable moisture relationships. In pastures where native white clover can be depended upon to supply nitrogen, response to complete mineral (calcium, potassium, phosphorus) fertilization will be pronounced.

In pastures where white clover cannot be depended upon to supply nitrogen, this element will be the first limiting element. Nitrogen will ordinarily be followed in turn by calcium (lime), potassium, and phosphorus. In such cases, a system of fertilization which includes nitrogen as well as complete minerals will be necessary to produce a pronounced response.

Reseeded Pastures

Reseeded pastures, in the sense discussed here, refer to areas which are tilled, fertilized, and reseeded to the more productive pasture species. Observations clearly indicate that before a potentially high yielding species, Ladino clover for example, will establish and maintain itself, a relatively high level of soil fertility must be provided. To attain such a fertility level in most crop soils in Massachusetts, it is necessary to incorporate fertilizer materials and organic matter into the soil in liberal quantities by some sort of tillage operations. This being accomplished, high yielding species can then be expected to produce pasture herbage commensurate with their potential yielding capacity.

Highly productive species will persist and remain in a high state of productivity just so long as it is possible to maintain a high level of soil fertility. With the aid of annual fertilizer top dressing applications, a relatively high level can be maintained in most instances from three to five years. To reattain the initial soil fertility level, it is necessary to repeat the tillage, fertilizer, and seeding practices. If reseeding operations are not carried out after three to five years, Kentucky bluegrass, native white clover, and various Agrostis species will become the dominant herbage plants.

The problem of herbage production in Massachusetts, in the opinion of the writer, centers mainly around soil fertility. If other factors, such as weather, physical soil conditions, and management practices, are reasonably favorable, not only the maintenance of particular species in many cases, but also the herbage yields of all species, are primarily functions of soil fertility. The struggle in Massachusetts is to provide a level of soil fertility which will maintain high yielding species, such as Ladino clover. Fertilizer top dressing alone will not accomplish this result. Tillage, fertilizers, organic matter, and seed are all important factors in establishing and maintaining productive species in productive pastures. If pastures are cared for and treated as any other high producing crop, they will yield in a comparable fashion.

New Hampshire

Trials in top dressing old pastures were continued in two locations during the past year. On the Seavey pasture, Greenland, New Hampshire, various nitrogen carriers are being tested alone and in complete fertilizers, superphosphate and potash are being used alone in triennial and annual applications, and nitrogen in nitrate of soda is being used at two levels. Half of each plot was limed at the outset.

Seven years' results are now available on this field. The principal response to date has come from nitrogen, although yields from the straight nitrogen plots appear to be on the decline. Superphosphate and potash alone stimulate the sward to some extent and in combination quite considerably, although when these materials are combined with nitrogen in a complete fertilizer they do not increase yields over nitrogen alone as much as their response when used alone would indicate.

Large triennial applications of superphosphate have proved slightly more effective than one third the amount used annually, but with potash the reverse has been true.

On the Livingston pasture near Claremont, New Hampshire, different nitrogen carriers and two levels of nitrogen have been used, superphosphate and potash have been used together, complete fertilizers have been compared and nitrogen and potash as well as nitrogen and phosphorus have been combined over a five-year period.

The quality of the herbage and midsummer yields are enhanced on all plots on which phosphoric acid and potash are included in the fertilizer. Potash appears to stimulate clover somewhat more than phosphoric acid. The use of superphosphate and potash together stimulates the sward more than the use of nitrogen alone and the results when these materials are used together are additive. The difference in response secured on this field as compared with Seavey pasture is due mainly to excellent clover wherever suitable materials are applied to encourage it.

In a study of pasture species in large plots for pasture and in small plots for harvesting, botanical analyses were made of the pastured plots for harvesting, botanical analyses were made of the pastured plots to determine which species survive to the greatest degree and harvests were made from the smaller plots to determine comparative yields.

Yield records were taken on twelve varieties of wild white clover, six grasses, and eleven mixtures of legumes and grasses. Among the white clovers, Wisconsin white headed the list as to yields, timothy led among the grasses, and a combination of alfalfa and orchard grass in the mixtures.

Samples of pure stands were taken during the season for chemical analysis.

New Jersey

(Dairy Department)

Pasture Fertilization in Relation to Carrying Capacity

The heifers on pasture were taken off the Wyker Farm October 22, which made a total of 164 pasture days for the season. All animals were weighed and measured before going on pasture and when taken off pasture. Animals removed in midseason were also weighed and measured. Forty-two of the fifty-nine animals turned on remained on pasture throughout the season.

The following data show the average number of 1000-pound cow-pasture days per acre for the years 1935, 1936, and 1937 on the Wyker Farm under rotation fertilization treatment, in comparison to the check plot which received no fertilizer treatment.

Year	All Wyker 1000-pound cow days per acre	Control Plot 1000-pound cow days per acre
1935	192.2	160.0
1936	225.2	143.9
1937	224.7	166.7

The first section of the data on page 114 show the results for all heifers turned on pasture, while the second section shows the results for heifers that remained on pasture all season. All figures are averages for the group.

No.	Total gains: No. days	Weight	Height	Heart girth	Daily gains: Weight	Height	Heart girth
Holstein 27	150.1	231.8	4.8	14.7	1.54	.032	.097
Guernsey 32	133.2	186.1	5.6	14.8	1.40	.042	.111
Holstein and Guernsey 59	140.9	207.0	5.3	14.7	1.47	.037	.105
Holstein 21	164	237.9	5.5	15.6	1.45	.033	.095
Guernsey 21	164	219.1	7.2	18.0	1.34	.044	.109
Holstein and Guernsey 42	164	228.5	6.3	16.8	1.39	.039	.102

Note: These gains in weight are at least 30 percent above those obtained last year.

Sod Improvement

Complete fertilization with lime continues to bring about the greatest sod improvement. Lime and potash produce a better clover-grass ratio than lime and superphosphate on the Duchess soils. Superphosphate alone has brought about no increase in clovers to date. The best coverage is produced by the complete basic fertilization plus nitrogen.

Herbage Changes

Herbage changes seem to be definitely tied up, not only with fertilization practices, but on good management factors. These management factors are close grazing, rest for regeneration, and clipping the areas.

Wiker field No. 2 has received elemental nitrogen up to 100 pounds per acre in the form of sulphate of ammonia, calcium cyanamid and nitrate of soda and urea annually since 1931. In addition to this nitrogen the pH, calcium, phosphorus, and potash needs of the soil have been met as far as possible. Contrary to the opinion of some authorities on the subject of pasture management, the clover-grass balance was improved at the start and has been maintained at a 25:75 percent ratio since that time. The clover-grass ratio is less disturbed by nitrogen than through management.

The close grazing, mowing, and resting of the sod prevents the grass from crowding out white clover.

Palatability

Manural application above 10 tons per acre applied late in the fall reduces the palatability of the grass for milking cows to the point that after a day on this pasture they will come into the barn without fill. This results in a terrific drop in milk production. It is much better to harvest this manured grass as grass silage or hay.

On the experimental plan used for the past season with the milking herd there did not seem to be any distinct palatability difference in herbage produced by the different forms of nitrogen.

Management in Relation to Cultural Practices

Through management, i.e., close grazing, rotational management, mowing aftermath, and spreading the droppings, the check field on the Wyker Farm experiment still continues to show improvement. The balance between clovers and grasses is improving. This particular experiment is becoming involved because there is fertility brought on to this plot by grazing animals. These animals graze grass from a well fertilized plot and then are moved on to the check plot. The manure deposited on the check plot by these animals from the fill of nutritious grass from the previous plot is high in fertility. This may explain some of the improvement that is being noted.

Close, Medium, and Light Grazing

Close grazing with alternate rest periods produces the best type of sod. Very close grazing with milk animals produces excellent sod but causes

a heavy drop in milk production. Medium grazing and close clipping the pasture after the removal of the animals is sounder grazing practice for milk production and no damage will be done to the sod.

Close grazing of a pasture in October or before the onset of a killing frost may injure the pasture area to a considerable extent if the area has large white clover patches. This is especially true if there is considerable freezing and thawing in the late winter and spring.

Grassland Management in Relation to Fertilization with Manure

Fertilizing permanent pastures with barnyard manure is an excellent method of improving soil fertility and stimulating yields of grass. However, it does depress the palatability of the herbage. To overcome this defect and still take advantage of the improvement it is advisable to cut this herbage early in June and preserve it in the silo with molasses.

Superphosphate in Hayland Improvement

Valley Farm field No. 7 was used to determine the effect of TVA superphosphate on the yields of timothy grass.

Four thousand pounds of TVA superphosphate were applied to the entire area of 13 acres on April 12 and 13, 1937. For a comparative study of yields 3 plots were laid out crosswise of the field. Plot No. 1 received 200 pounds of TVA superphosphate per acre, plot No. 2, 250 pounds per acre, and plot No. 3, 300 pounds per acre.

On June 18 and 19, 1937, the green hay was removed from these plots. At the time of cutting the mower was stopped and all grass cleaned from the cutting bar. Then cutting was resumed for a distance of six feet. Since the machine cuts a swath six and one-half feet wide, this made an

area of 39 square feet. The cut grass from this area was carefully raked and weighed, moisture determined, and a representative portion taken for analysis from each sample. Five such samples were taken in each plot, and the acre yields determined from each sample.

The following data show the results for this year:

V. 7, TVA Superphosphate Plots

Date of application	Amount per acre	Yield per acre	Percent moisture	Dry matter per acre
April 12	200 lbs.	17,179.28	72.8	4,672.76
April 13	250 lbs.	16,834.05	71.6	4,780.87
	300 lbs.	15,703.06	73.4	4,177.01

Since past experience shows that true values cannot be obtained with a one-year study of yields on superphosphate applications, we plan to continue this experiment further, securing yields and analyses in the same manner during 1938 and succeeding years, which will give a more complete picture of the various applications.

Economy of Rotational Grazing on the Wyker Farm Area

After applying all charges of interest, depreciation, labor, and cost of fertilizers to the Wyker Farm grazing experiment consisting of 48 acres, one finds this system of management an economical way of raising replacements. The only cred applied to the area was for hay harvested and sold to production units at $15.00 per ton. After deducting this credit from the charges it was found that the replacements were grown on pasture at a cost of $ .021 a pound gain. The average daily gain was 1.47 pounds per animal.

(Agronomy Department)

Response of Permanent Pasture Sod to Fertilization and Liming

A 3-year study on the response of a permanent pasture sod to various soil treatments showed an increase of 47 percent in total growth for the LPK treatment, 74 percent for LNPK, and 88 percent for LNPK plus nitrogen in June. The effect of March applications of nitrogen was expressed mostly prior to June 15; after that date to the end of the season the LPK treatment showed a substantially greater increase than LNPK. June application of nitrogen increased growth above the level of LPK until mid-August but failed to maintain equal growth thereafter. Both the LPK and LNPK substantially increased growth over the checks at all seasons, with the LPK showing the most uniform seasonal improvement. Clover was greatly increased by lime and minerals to an average of 36 percent of all vegetation. March application of nitrogen reduced clover to 14 percent, and March plus June nitrogen reduced clover to 60 percent.

The total cost of producing a ton of dry clippings was $13.76 for no treatment, $12.60 for LPK, $16,15 for LNPK, and $17.78 for LNPK plus nitrogen. All treated plots produced herbage averaging 18 percent protein. Since these costs are considerably lower than the cost of purchased feeds of equal quality, it is obvious that pasture improvement is profitable. If a correction is made for palatability, the treatments would be considerably more valuable than indicated above. On pastures of limited acreage in proportion to stock, heavy nitrogen feeding would be most profitable; on more adequate acreages, the LPK treatment would have the advantage. On many farms, the two systems might well be used on different acreages to supplement one another. Because of peak growth in May and June following March applications of nitrogen, harvest of surplus feed as hay or silage would be necessary to use the increased feed produced.

Comparisons of nitrogen alone, LPK and LNPK treatments with checks

on 25 permanent pastures throughout the state, indicate a favorable response to both LPK and LNPK treatments. Nitrogen alone failed to increase feed after early spring and did not improve the vigor of the sod. The value of LPK treatments depends in large measure on the stimulation of clover. In some cases clover does not increase to a satisfactory level until the second year after treatment. There is some evidence that the second application of minerals is more effective than the first in stimulating clover. Fifty pounds of nitrogen provided as either nitrate or ammonia definitely suppressed clover, although on some pastures clover percentage was greater on LNPK plots than on checks. The average percent clover for 25 pastures was as follows: No treatment, 4 percent; nitrogen alone, 4 percent; LNPK, 10 percent; and LPK, 38 percent.

In 1938, a year of favorable moisture supply, a cooperative pasture on Penn shale loam showed 75 percent increase in clippings from nitrogen alone, 118 percent increase from LNPK, 175 percent from LPK, 66 percent from LNP, and 41 percent from LP. There was 22 percent clover on LNPK and 66 percent for LPK, in contrast to 3 percent for the check.

Palatability

In grazing trials on 22 pasture mixtures on which 8 types of soil treatments were applied, the preferences of dairy cows have been noted throughout the season for several years. The relative palatability of the principal species which have survived in these mixtures are: first, clover; second, timothy; third, orchard grass; fourth, Kentucky bluegrass; fifth, red top. Conflicting reports were obtained on Canada bluegrass. It was significant that clover was palatable in all stages of growth, and that all grasses became unpalatable at the stage when seed heads were being formed. Since the various grasses produce seed heads at different times, care is required to make accurate estimates of palatability.

During the early part of the season, plots receiving nitrogen fertilizer plus LPK were preferred by grazing cattle. After June 15 when the apparent effect of the nitrogen had largely disappeared, there was no superiority in palatability over the LPK plots. In general, the use of lime and minerals substantially increased the palatability over check plots. On the 25 small plot cooperative tests distributed through the State, striking evidence on palatability was obtained; the treated areas were grazed much more satisfactorily than untreated surrounding areas. Palatability was maintained most satisfactorily throughout the season by treatments which increased clover to a substantial level.

Types of Phosphate Fertilizers

In the detailed studies on various types of phosphate fertilizer, applied to a single soil type, calcined phosphate alone produced as great increases as lime and superphosphate. Calcium metaphosphate and di-sodium phosphate were equal to superphosphate when both were accompanied by lime. Calcined phosphate produced excellent results without lime, whereas the other phosphates produced little response unless accompanied by lime. Calcium silicate (quenched) from the TVA, produced results comparable to LPK, presumably because of the beneficial effect of the silica on the availability of natural soil phosphates. In cooperative tests, on various soil types, calcium silicate did not show such favorable effects, possibly because the material had not been ground sufficiently fine.

Breeding White Clover

A breeding project with white clover has been under way with special observations on the variability within different commercial sources of seed and within 15 native strains collected in New Jersey. A great range of type was found within the native strains, varying in many cases from the ex-

tremely large type characteristic of Ladino to the extremely dwarf type characteristic of the Kent strain. Great differences were observed within strains in leafiness, density of growth, spread of the plant, length and diameter of stolons, height of petiole, and similar characters. Kent and Ladino were the only strains with distinct morphological types. The other strains showed greater variability in the various characters than native strains, but the extremes were fully as wide in the latter. Since native strains presumably are better adapted to local conditions of soil and climate, selection for desired types might well take place within such populations. Certain characters were generally associated, such as wide spreading habit, long internodes, thicker stolons, taller petioles and flower stalks, and larger leaflets. Such giant types occurred in all populations except the Kent.

In studies on the value of various types of clover introduced in a uniform grass mixture, the Ladino seemed fully equal to Kent in the total yield, both when clipped regularly every two weeks and when harvested at the time growth had reached a height of 5 to 6 inches.

In a comparison of Wisconsin and Ladino clover seeded on 25 cooperative pastures in conjunction with various fertilizer treatments, both strains were satisfactorily established by broadcasting seed in March without any tillage of soil. The Ladino failed to survive during the ensuing 2-year period, under conditions of grazing given these cooperative pastures. The addition of Wisconsin clover seed did not appreciably increase clover percentages over those on similarly treated plots where native clover came in spontaneously. Apparently Ladino clover must have special management in order to be of real value in permanent pastures.

Greenhouse studies on differential mineral absorption by commercial strains indicated superiority of the Wisconsin strain for obtaining potash

on a deficient soil. Kent clover was apparently superior to Ladino in feeding power for potash. No apparent differences were noted for phosphates and lime, except that the Louisiana strain showed greater root growth in acid, phosphate deficient soil.

New York (Cornell)

Pasture research was continued at Cornell University in 1937-38, and further information was obtained concerning the seasonal productivity, chemical composition, and persistence of various species and strains of grasses and legumes when grown alone and in association under conditions of periodical close grazing. The results obtained since 1931 indicate that the following species and varieties of grasses and legumes are of special value on soils adequately fertilized for inclusion in mixtures intended for the establishment of permanent pastures which will be periodically closely grazed:

Grasses

Species	Varieties or Strains	Country of Origin
Perennial ryegrass (*Lolium perenne*)	Svalof Victoria	Sweden
	Jaedrsk	Norway
	E. F. 79	Denmark
Timothy (*Phleum pratense*)	Aberystwyth Pasture S. 50	Wales
	Corstorphine Pasture C.B. 191	Scotland
	Aberystwyth Pasture-hay S. 48	Wales
Kentucky bluegrass (*Poa pratensis*)	Commercial	U.S.A.
	Øtofte	Denmark
	Aberystwyth S. 63	Wales
Rough stalked meadow grass (*Poa trivialis*)	Commercial	U.S.A.
		Denmark
Canada bluegrass (*Poa compressa*)	Commercial	U.S.A.
		Canada
Orchard grass (*Dactylis glomerata*)	Svalof Brage	Sweden
Meadow fescue (*Festuca elatior*	English wild	England
	Aberstwyth S. 53	Wales
Meadow foxtail (*Alopecurus pratensis*)	English wild	England

Legumes

Species	Varieties or Strains	Country of Origin
White clover (*Trifolium repens*)	Kent wild New York wild	England U.S.A.
Bird's foot trefoil (*Lotus corniculatus*)	New York wild	U.S.A.
Yellow trefoil (*Medicago lupulina*)	Commercial	England

Experiments are in progress to determine the value of various species and strains of grasses and legumes for inclusion in mixtures intended primarily for the production of hay or silage and aftermath grazing.

Investigations have been continued to determine the influence of wild white clover on the productivity and chemical composition of Kentucky bluegrass, and upon soil temperature, water conservation, and the earthworm population of the soil. In 1935, a mixture of 24 pounds Kentucky bluegrass and 2 pounds wild white clover per acre seeded in 1933 yielded 4985 pounds of dry matter per acre, averaging 31 percent of protein. The mixed herbage contained 2243 pounds of Kentucky bluegrass, averaging 25 percent protein, and 2742 pounds of wild white clover, averaging 35 percent protein. The yield of Kentucky bluegrass seeded along at the rate of 24 pounds per acre was 888 pounds of dry matter averaging 18 percent of protein. The association of wild white clover with grass has reduced the loss of water by run-off from the surface and has led to an increase in the earthworm content of the soil. Soil temperatures under a sward of Kentucky bluegrass and wild white clover have been maintained at a lower level during the summer months than under a sward of Kentucky bluegrass alone. During the peak period of growth on a day in late May, the soil temperature at one inch below the surface under Kentucky bluegrass alone ranged between 40 and 73 degrees Fahrenheit, whereas under a plot of Kentucky bluegrass and wild white clover, it ranged between 47 and 68 degrees.

Experiments on the fertilization of New York pastures have been continued and have led to the following conclusions:

1. Most New York pastures show a marked response to heavy applications of superphosphate.

2. Lime, in addition to phosphorus, is usually beneficial on soils testing below pH 6, and it is frequently essential on soils testing below pH 5.5.

3. Potash is seldom required in addition to phosphorus and lime for satisfactory pasture improvement. Occasionally a marked response is obtained on the lighter soil types or in fields which have been depleted in potash by the growth of hay or other crops.

4. Nitrogen can be supplied most effectively and most economically by the encouragement of pasture legumes, especially wild white clover. Other legumes which are considered of value in permanent pastures under New York conditions are yellow trefoil and bird's foot trefoil.

During 1937 the influence of fertilizer treatment with 800 pounds of 16 percent superphosphate, 200 pounds muriate of potash, and 2000 pounds ground limestone per acre, on the seasonal productivity of a poor pasture at Cornell University, was measured by removing all herbage with a lawn mower at intervals during the grazing season. The following results were obtained.

Period	Dry matter per acre per day	
	Untreated plot	Plot treated with P, K, and Ca
April 21 - May 21	2	15
May 22 - June 11	5	33
June 12 - July 10	5	16
July 11 - August 6	6	20
August 7 - August 24	6	27
August 25 - October 1	1	15

The total yield of the treated plot was 3227 pounds of dry matter per acre, averaging 20.2 percent protein, compared with 629 pounds of dry matter, averaging 12.8 percent protein on the untreated plot.

Volusia silt loam from a poverty grass pasture in Cortland County was used in an experiment to determine the response to fertilizer treatment of poverty grass (Danthonia spicata), devil's paintbrush (Hieracium aurantiacum), Kentucky bluegrass (Poa pratensis), and wild white clover (Trifolium repens), when grown alone and in association with each other. The results indicate that each species is benefited by treatment with phosphorus, potash, nitrogen, and lime, but that the percentage increase in yield as a result of treatment is considerably greater for wild white clover and Kentucky bluegrass than for devil's paintbrush and poverty grass. When the plants are grown in association, wild white clover and Kentucky bluegrass tend to suppress devil's paintbrush and poverty grass under the influence of fertilizer treatment. When not fertilized, devil's paintbrush and poverty grass tend to suppress wild white clover and Kentucky bluegrass.

The results of pasture research at Cornell University and in other parts of New York State are included in the following publications:

D. B. Johnstone-Wallace - Cornell University Agricultural Experiment Station Bulletins-
No. 538, Part II, Pastures in Cayuga County
No. 567, Part II, Pastures in Genesee County
No. 570, Part II, Pastures in St. Laurence County
No. 600, Part II, Pastures in Nassau and Suffolk Counties
No. 612, Part II, Pastures in Herkimer County
No. 630, Part II, Pastures in Erie County
No. 639, Part II, Pastures in Delaware County.

D. B. Johnstone-Wallace - "The Influence of Grazing Management and Plant Associations on the Chemical Composition of Pasture Plants." Jour. Am. Soc. of Agron., Vol. 29, No. 6, June 1937.

D. B. Johnstone-Wallace - "The Influence of Wild White Clover on the Seasonal Production and Chemical Composition of Pasture Herbage and upon Soil Temperature, Soil Moisture, and Erosion Control." Report of 4th Interntl. Grassland Congress, Aberystwyth, Great Britain, December 31, 1937.

D. B. Johnstone-Wallace - "Mixed Cropping and Excretion of Nitrogen by Leguminous Plants." Discussion of paper by P. W. Wilson and Orville Wyss. Proc.Soil Sci.Soc. of Am., Vol. II, 1937.

D. B. Johnstone-Wallace - "Pasture Improvement and Management". Cornell University Extension Bul. No. 393, October 1938.

H. A. MacDonald - "A Study of the Effect of Fertilizer Applications on Change of Botanical Composition of Pasture Swards." Thesis presented to the Faculty of the Graduate School of Cornell University for Degree of Master of Science. June 1938.

Pennsylvania

Pasture Fertilization

This experiment, now in its tenth and last year at the present location on DeKalb soil in Clearfield County, has yielded results in line with those previously reported. The lime phosphorus treatment continues to be the most profitable under average farm conditions. The grazing results for the season of 1937 are as follows:

Treatment	Total pasture days per acre	Total production (pounds) 4 percent milk per acre
Lime	29	1107
Lime and P_2O_5	75	2134
Lime, P_2O_5, and K	80	2860
Lime, P_2O_5, K and N (24 pounds)	82	2270
Lime, P_2O_5, potash, and N (36 plus 36 pounds)	113	3648

Rejuvenation of Worn-Out Pastures

In an attempt to rejuvenate an old worn-out pasture, a plot of ground was limed(4,000 pounds) and fertilized with 1000 pounds 20 percent superphosphate and 250 pounds muriate of potash in March 1938. Inoculated sweet clover seed was then sown and cultipacked in at the rate of 20 pounds per acre. Three thousand two hundred sixty-five pounds of green material were harvested in August 1938. The main object is to determine whether the nitrogen accumulated by the sweet clover will tend to stimulate a good stand of

grass.

Testing and Breeding Strains of Clover

Series tests on local, foreing, and American strains of red clover conducted in the past year indicate that local strains are superior to anything else. Foreign strains produced a sufficiently good crop to justify their use when good domestic seed is not available.

The breeding work with red clover has continued along the same lines, as previously reported. Additional inbred families have been produced for inheritance studies and the possible synthesizing of new varieties.

Testing and Breeding Grasses for Pasture and Other Uses

Referring to the report submitted on this project one year ago, it is of interest to report that about 80 strains of Kentucky bluegrass and about 40 strains of timothy are being worked on at the present time. No data have been published to date.

The Effect of Different Systems of Grazing, Cutting, and Fertilization On White Clover in Permanent Pastures

A description of this project was reported one year ago. One season of work has been conducted with various strains of clover on a study of changes of population that occur from the beginning to the end of the grazing season. About 14,000 items of data have been accumulated to date. Aamodt's modified point quadrat method is being used with very satisfactory results.

Growth and Maintenance as Influenced by Various Soil, Fertilization and Methods of Turf Management

Referring to the report submitted a year ago, it is of interest to mention that new work has been started to determine the value of peat

materials and by-products of tanneries as sources of organic matter in the maintenance of golf greens. Other new phases of study deal with the rate of seeding Kentucky bluegrass, time and height of cutting, and value of different depths of top soil.

The use of organic and mixtures of organic and inorganic nitrogen compounds has proven more desirable in the growth of grasses than where all of the nitrogen is supplied in immediately soluble form. It is the tendency for the latter to produce excessive growth during the early months of the year, whereas the organic nitrogen materials produce more uniform growth throughout the season. Bone meal and superphosphate have given about equally good results.

The results of these investigations have been reported at the annual meeting of greenkeepers for the past three years. No data have been published to date.

Rhode Island

Response of Pasture Grasses to Environmental Factors - Flowering

During the winter of 1937-38 studies were made on the effect of day-length on the growth habits of twelve grass species commonly found in the ecological population of northeastern pastures or being recommended for seed mixtures for pastures. These grasses were: Kentucky bluegrass, Canada bluegrass, colonial bent, velvet bent, red top, meadow fescue, Italian ryegrass, perennial ryegrass, commercial timothy, timothy S-50 (a selected strain), orchard grass, and rough stalked meadow grass. These grasses were grown in wooden flats in the greenhouse where the temperature was common to all and also recorded. The moisture conditions and soil nutrients were also optimum with all grasses. A complete fertilizer was added at the beginning of the experiment and additional nitrogen was added monthly. Additional light was supplied by the use of 100-watt bulbs sus-

pended over the flats so that day length conditions of eight and sixteen hours were secured. The period of growth was from November 1, 1937, to April 1, 1938. Growth was measured by the weight of clippings taken weekly at $2\frac{1}{2}$ inches. Observations were recorded during the period on the date of flowering and growth habit. Control flats, both clipped and unclipped, were maintained under the varying day lengths with no additional light supplied.

The plants exposed to the sixteen-hour day grew more rapidly at the beginning, but the total yields of clipping was no greater than that of the plants exposed to the eight-hour day or the checks. The habit of growth was strongly vertical, being especially noticeable in the clipped plants. The unclipped plants tended to grow very tall, but were not sturdy enough to stand erect without support. The sixteen-hour day plants had consistently somewhat narrower, longer leaves and were paler and brighter in color but not chlorotic.

All of the plants exposed to the eight-hour day showed a horizontal habit of growth as contrasted with the vertical growth of the sixteen-hour day plants. The leaves tended to be broad and thick and of a dark green color.

The controls, both clipped and unclipped, resembled the short-day plants in habit and appearance during the winter months but in the spring when the days became longer, the habit of growth tended to become more vertical but never so pronounced as that of the long-day plants.

No conclusive data were obtained as to the influence of day length on flower development. There are indications that Canada bluegrass, orchard grass, Italian and perennial ryegrass, commercial timothy, and meadow fescue may respond to critical day lengths. A critical analysis of the data and the other growth factors leads to the opinion that the temperature at which the plants were grown may be as great a factor as inducing flowering as day length.

Moisture Relations

Kentucky bluegrass, colonial bent, and timothy S-50 were grown during the summer months of 1938, in rectangular enameled pans approximately 10 x 17 x 4 inches in soil in which a complete fertilizer had been mixed. Moisture levels of 25 percent and 15 percent were maintained by daily weighing of the pans and contents and making up to weight by the addition of water. The grasses were clipped at $2\frac{1}{2}$ inches periodically. Observations of growth were recorded both during and at the end of the experiment.

Both colonial bent and Kentucky bluegrass were more vigorous when grown with 25 percent moisture as shown by weights of clippings. Stolons were more numerous and new roots more profuse under the high water conditions. The total root volume of Kentucky bluegrass appeared greater when 25 percent soil moisture was supplied. The rootlets were more numerous at the lower water level. Colonial bent appeared to have little difference in total root volume in the two moisture conditions, but, as with Kentucky bluegrass, the branching of the rootlets was more profuse with the lower soil moisture. The yield of clippings shows that timothy S-50 grew better with 25 percent moisture during the first month of the experiment. For the remainder of the experiment there was little growth in either series. At harvest the low moisture plants had much better developed root systems than those of plants grown with 25 percent moisture. There were very few stolons on the high water plants and none on the low water plants.

Graphical comparisons of the curves of clipping weights of both high and low water yields of all three grasses show that even with 25 percent soil moisture there was a decided decrease in growth during July and August. It would appear that moisture as such is not the limiting factor responsible for the slower growth rate of certain pasture grasses during midsummer.

Culture Methods of Supplementary Pasture Crops

Field experiments were begun during the spring of 1938 to secure information on the adaptability of certain crops for supplementary pasture in Rhode Island. The crops included are Sudan grass, Japanese millet, oats, winter rye, and winter wheat. Data are being obtained on rates and dates of seeding, seed bed preparation, dates when available for grazing, amount of material produced, and chemical composition.

Rye seeded in the fall of 1937 furnished material for grazing from April 5 to May 10. Permanent pastures can be used from this date to about July 1. A total yield of about 5 or 6 tons of green rye per acre was produced.

Oats seeded April 15 furnished material for grazing from June 15 to July 15. A total yield of about 5 tons per acre was produced. Second crops of hay meadows can be utilized from July 1 to August 1, also.

Sudan grass planted at different intervals from June 1 to July 15 furnished grazing material from July 15 to September 15. A total yield of about 6 to 8 tons was furnished in three cuttings made from July 15 to September 1 on Sudan grass planted June 1. Sudan planted in a firm seed bed yielded about three times that planted in a loose seed bed. This seems to be a major consideration in obtaining a satisfactory stand of Sudan grass. A heavier seeding than is ordinarily used was also found to yield much better returns. When the Sudan grass was cut at a one-inch height, more total yield was obtained in three cuttings than when it was cut at 2-, 3-, or 4-inch heights. In other words, it thrived best under conditions simulating close grazing.

Vermont

Relative Value of Four White Clover Strains Under Pasture Conditions with Different Fertilizer Treatments

Results from this study have been published in Vermont Station Bul-

letin 431, "Four Pasture Clovers", March 1938. They show that native white clover readily established itself on three different soil types when properly fertilized and managed. This clover was much more persistent than the others, i.e., common white Dutch, Ladino, and English kent, and seemed to survive greater extremes in grazing management. Ladino clover is more of a hay type plant and does best when not kept grazed too closely; while English kent does best when closely grazed. Wild clovers naturally "come in" on Vermont pastures when properly handled; therefore, it is not necessary to go to the added expense of plowing and seeding to obtain good permanent pastures.

Nitrogen vs. Clovers for Pasture

The object of this experiment was to study the relative merits of these two types of pasture. Some difficulty has been experienced in this work because orchard grass was used in the original seeding. This grass makes a very early, rank growth and gives the low-growing clover too much competition in the early spring. Then too, it has been difficult to obtain enough grazing animals to properly control the grazing on these plots at the U. S. Morgan Horse Farm.

Pasture Seeding Mixtures

Yields and palatability of different pasture and hay plants have been studied. While there are differences in the palatability between the different grasses used, yet, when they are kept in the vegetative state, they are all relished by cattle and sheep. Orchard grass grows very early and offers considerable possibility for early pasturage, especially nitrogenous fertilizers, but it cannot be successfully used on permanent pastures where clovers and low-growing grasses are desired unless it is kept heavily grazed in early spring.

Value of Farm Manure on Permanent Pastures

Manure, with and without superphosphate, has been compared with different fertilizers containing nitrogen. The results indicate that superphosphate greatly increases the value of manure. While natural white clover can be maintained with treatment, yet care must be exercised with the use of manure on permanent pastures because animals do not readily eat vegetation that has been recently manured. In this way, it may stimulate grass and give the clover too much competition.

Fall vs. Spring Applications of Nitrogen on Pasture and Meadow Land

The results show that very little nitrogen is lost when applied in late fall on a good turf. This is especially true with cyanamid and urea. While spring applications usually give slightly greater yields than where the nitrogen is applied in the fall, yet the latter method may be desirable since it is usually more convenient to apply at this time.

Some "burning" was observed with cyanamid, even with the fall applications. This was probably because it was too cold for proper chemical and biological changes in the cyanamid to take place. Both fall and spring applications of nitrogen were materially aided by use of some added phosphorus and potash. Both quality and quantity of vegetation was greatly improved with these added minerals, and it is evident that meadows can be maintained for long periods of time in a high state of productivity without the expense of plowing and reseeding, if properly fertilized.

Contributions to Pasture Research
July 1, 1937, to June 30, 1938

Midgley, A. R. "Four Pasture Clovers." Vt. Sta. Bul. 431, March 1938.

Midgley, A. R. "Permanent Clovers for Permanent Pastures". Better Crops with Plant Food Magazine, Vol. 22, August 1938.

West Virginia

Small Plot Experiments

Yield data and botanical estimates showing residual effect of fertilizers and lime on different soils in four locations continue to show that phosphorus and lime are the principal limiting factors. The increase in yield on the plot receiving lime and superphosphate over the limed plot varied from 13 to 43 percent in the various areas. Increases for lime varied from 0 to 12 percent. Some of the plots showed a slight increase as a result of the application of potash fertilizer. Nitrogen showed an effect on all plots, the most marked effect being noted on those plots which had only a small percentage of legumes. In addition to the effect on yield the fertilizer and lime treatments brought about an increase in the content of desirable pasture plants.

In the spring of 1937 some of the check plots were treated with lime and superphosphate and a few had seed in addition. The addition of lime and 500 pounds of 20 percent superphosphate increased the desirable species from 2 percent in the untreated plots to 15 percent in the treated plots. Seed in addition to lime and superphosphate increased the percentage of desirable species to 23 percent, most of the increase being the result of additional clover. The yield of forage was increased by about 25 percent, there being very little difference between the plots which had seed and those which did not.

Grazing Experiment

These plots continue to show a correlation between yields obtained by clipping and grazing. The yield of total digestible nutrients based on 100 for the grazing method were 67 for the permanent plots, 75 for plots relocated each year, and 83 by the difference method.

Time of Nitrogen Application to Pastures

Nitrate of soda applied at the rate of 200 pounds per acre gave an increase of 47 percent in the yield of clipped herbage. There was very little difference in total yield as a result of different times of application. However, on those plots which received part of the nitrogen in June or July the production was higher during the latter part of the season. The data for this experiment have been summarized and will soon be submitted for publication.

Residual Effect of Lime and Fertilizers

Lime applications in 1923 and superphosphate in 1927 are still showing a marked residual effect on the botanical composition of an old established pasture. There are only 10 percent desirable species on the untreated plots as compared to 34 percent on the limed plots and 41 percent on those receiving lime and superphosphate. Very little difference was evident in the yield of clipped herbage of the various plots. Soil samples taken in 1937 show that the lime has penetrated about 7 inches, but the effect is most marked near the surface.

Persistence and Growth of Different Strains of White Clover in Permanent Pastures as Affected by Various Practices

The results of the first year show a marked decline in white clover when cut twice during the season at the hay stage. Clipping three inches high in the spring followed by $1\frac{1}{2}$-inch clipping during the latter part of the season has also reduced the clover content as compared to $1\frac{1}{2}$-inch clipping over the entire season. No difference was noted between the three strains tested.

Lespedeza in Pastures

A very poor stand was obtained in permanent pastures. In some plots clipped with a lawn mower the lespedeza plots gave higher yields during the latter part of the summer than those plots which did not have lespedeza.

Publications

R. R. Robinson and W. H. Pierre, "Response of Permanent Pastures to Lime and Fertilizers (1930-1936)", W. Va. Agr. Expt. Sta. Bul. 289.

Appendix B

REPORT OF COLLABORATORS' MEETING

The second annual meeting of collaborators with the U. S. Regional Pasture Research Laboratory was begun at nine o'clock on Wednesday morning, September 7, with all collaborators present except representatives from New Hampshire and West Virginia. In addition, the Office of Experiment Stations and the Division of Forage Crops and Diseases were represented at the meeting. The sessions continued more or less intermittently until the next day at 4 p.m. when the meeting was adjourned. Practically the entire time was given over to reviewing the research work carried on at the Laboratory during the past year, and that contemplated in the immediate future. A short time before adjournment, written suggestions in the form of committee reports were approved as a guide to bring about a more fully coordinated research program between the Laboratory and State agricultural experiment stations in the Region. Tentative plans were made to give particular attention, at the next annual meeting of the Collaborators, to pasture research at the State Stations, and to ways and means of effecting closer cooperative relations among State stations in the Region and between these stations and the Laboratory. Since the Laboratory's research has been so recently organized, its formative program has naturally come in for a major share of the Collab-

orators' attention, but this situation should not cintinue once the main lines of research to be carried on by the Laboratory are determined. A chronological and more detailed account of the meeting follows.

In opening the meeting, the Director of the Laboratory gave a brief report of the progress made during the year in attaining the general objectives of the Laboratory. The changes in perconnel, the initiation of plant pathological investigations, the additional equipment including a new greenhouse one hundred and twenty-two feet by thirty-five feet were mentioned. Emphasis was placed on the activities carried on to foster closer cooperative relations in the Region. In this connection were mentioned the exchange of project outlines between the State stations and Laboratory, the professional visits of State station workers at the Laboratory and vice versa,and the meeting of plant breeders interested in pasture improvement in the northeastern States, held in New York City last March.

Following this general introduction, J. T. Sullivan presented a detailed account and some data relative to the biochemical investigations under way at the Laboratory. A white clover plant has been found which tests 500 parts per million of HCN by a method used by other workers with Sudan grass, but the fact that this plant did not kill a young sheep when it grazed the plant might suggest that the test is not specific for HCN. About 75 percent of the plants tested showed no HCN. A chemical study on total nitrogen of one hundred thirty-seven individual plants of Kentucky bluegrass showed a range of 1.4 to 2.6 percent total nitrogen in the leaves. A brief description was given of an experiment to build up various levels of plant food stored in the underground parts of Kentucky bluegrass and Canada bluegrass.

V. G. Sprague described some physiological studies under way and results obtained thus far. It has been found that exposing soaked seed of Kentucky bluegrass to a temperature of 7°C. for a short time is effective in promoting germination. Supplementary light from Mazda lamps was found ef-

fective in inducing heading in Canada bluegrass, but such treatment in itself did not effect heading in Kentucky bluegrass. A preliminary vernalization experiment with several grass species gave results of interest and merits further investigation. A mechanism was described by means of which plants could be grown in fine gravel supplied at intervals with nutrient solutions. A small air pump was used as the agency for forcing the solution into the gallon jars containing the gravel. Six clones of Kentucky bluegrass growing under these conditions showed a differential response to different amounts of nitrogen and phosphorus in the nutrient solutions. It is planned to use a similar device to study water requirements of certain pasture plants.

R. R. Robinson described plans for making a similar study but by different methods and with the plants grown in soil. Yield data were presented from twenty-nine different clones of white clover representing divergent types grown in gallon jars filled with soil limed to two different pH levels and treated with two different quantities of superphosphate. The clones showed a marked differential response to lime and phosphatic fertilization, thus suggesting that clovers may be bred for different levels of soil productivity and acidity.

S. J. P. Chilton, who joined the staff last July, described briefly plans for undertaking plant pathological investigations at the Laboratory.

W. H. Brittingham gave a progress report on studies of the nature and extent of apomixis in bluegrass. He found that seed set under parchment bags was reduced in Kentucky bluegrass but that practically all plants did set seed. In Canada bluegrass, seed set under bags was poor and only about twenty percent of the plants set any seed at all. Progenies from open-pollinated seed and seed produced under bags will be planted adjacent to clonal increases of the parental plants to determine the extent and nature of apomictic reproduction in Kentucky bluegrass.

W. M. Myers discussed cytogenetic investigation of pasture grass species other than the Poas and grass breeding. Colchicine was found effective in producing chromosome doubling in rye grass. A considerable range of self fertility has been found in ryegrass, timothy, and orchard grass. Hot water has been found effective as a depollinating agent in timothy. Sod plots from individual plants of Kentucky bluegrass, Canada bluegrass, orchard grass, timothy, and Agrostis have been established by clonal increases and are being studied as a first step in an attempt to evaluate pasture types.

S. S. Atwood described the cytogenetic and breeding work under way with pasture legumes, particularly white clover. Sod plots from sixty-four divergent types of white clover have been established in a similar manner and for a similar purpose as the sod plots of the grasses. Plans are under way to add sod plots of Ladino during the next year. A wide range in self fertility and some interesting genetic characters have been found in white clover. Colchicine has been found effective in producing chromosome doubling both in white clover and red clover. Some first and second generation inbred lines of white clover have been produced. A beginning has been made in establishing inbred lines of Sudan grass.

The first day of the meeting was required for presentation of reports of progress of research at the Laboratory and the general discussions incident thereto. At the evening session plans were discussed for initiating cooperative efforts between the Laboratory and the State stations in the development of improved strains of pasture plants. One plan was discussed using white clover for illustrative purposes. No decision was reached, but the matter was discussed further in committee meeting the following morning and some general recommendations made. Another matter that was brought up at the evening meeting was the advisability of including in the annual report of the Laboratory a progress report of pasture research at the several State stations. It was recommended that this be done and that each collaborator be responsible for assembling the report of his particular station.

On Thursday morning the collaborators divided themselves into two committees to study more intensively some of the research under way at the Laboratory and to make recommendations. While the committees at the Laboratory were meeting, C. E. F. Guterman reviewed the research in plant pathology at the Laboratory as proposed by S. J. P. Chilton.

At eleven o'clock in the forenoon the collaborators reassembled to consider the committee reports. Dr. Guterman commented informally on the proposed research in plant pathology, pointing out the necessity of doing some survey work to ascertain the incidence of various diseases of pasture plants and their relative importance. He also stressed the desirability of studying the damage caused by insects and possible control measures. The most promising method of controlling plant diseases in pasture is by breeding for resistance.

The committee reports which were adopted unanimously follow:

Physiology and Soils

1. We reiterate the opinion of the committee of last year; namely, that the Pasture Research Laboratory should not be concerned with palatability. The palatability of any strains produced at the Pasture Research Laboratory should be tested at the State stations at the discretion of the workers in the States.

2. That the Pasture Research Laboratory attempt to determine the effects of controlled, constant, and varying temperatures and moisture levels on pasture plants.

3. It was agreed that the soils work at the Pasture Research Laboratory be concerned principally with testing the effects of varying levels of fertility and moisture on the strains of pasture plants evolved at the station.

Cytogenetics and Breeding

1. The collaborators recognize the value of the pasture plant breeding activities which are now being carried on at the State stations, and strongly recommend that these activities be continued and wherever possible enlarged. The laboratory should continue to give major attention to the accumulation of factual knowledge underlying methods of breeding.

2. It is also recommended that plans be developed to facilitate the ready exchange of plant material produced in connection with the respective programs carried on at the State stations and at the Laboratory.

3. In line with the above statements, it will be advisable for the individual State stations to maintain a testing nursery to evaluate any new strains produced anywhere in the Region to determine their local value.

From one-thirty to three on Thursday afternoon, the collaborators inspected the nursery. (The previous day a short time had been taken to examine some of the plant cultures and experimental devices set up in the greenhouses.) Before adjournment the group reassembled in the conference room for ice cream refreshments donated with the compliments of the Dairy Department of the Pennsylvania State College. At this final gathering, plans were discussed for the next annual meeting. It was suggested that more time be given to a consideration of the pasture research in progress at State stations. The details for working out a program for the next annual meeting were left to the Referee for the Northeastern Directors and the Director of the Laboratory.

Appendix C

REPORT OF NORTHEASTERN PLANT BREEDERS' CONFERENCE

In accordance with a suggestion made at the first annual meeting of the U. S. Regional Pasture Research Laboratory collaborators, a meeting of plant breeders interested in pasture plants was held in New York City, March 18 and 19, 1938. Representatives were present from Maine, Maryland, Massachusetts, New Hampshire, New Jersey, Pennsylvania, Rhode Island, and the Division of Forage Crops and Diseases, both from the Washington Office and the Regional Laboratory. In all, nineteen persons attended the conference which lasted a day and one-half.

The primary purpose of the meeting was to discuss informally problems and methods of attack and to plan a well coordinated program of pasture improvement by breeding. In this connection a clarification of the proposed functions of the Laboratory was sought.

No attempt was made to keep a verbatum record of the discussion but notes were made and from these notes this report was prepared. The plan of procedure followed in the meeting was to have a topic introduced by a discussion leader and then the topic thrown open for general discussion by the group. In this report the topics are taken up more or less in the order in which they were considered in the conference.

1. The function of the Regional Laboratory in a breeding program.

Discussion Leader: W. G. Colby, Mass. Agr. Expt. Station.

Colby suggested two general lines of attach which the Laboratory might follow in a breeding program: (1) the practical improvement of adapted grasses and legumes, and (2) more theoretical studies in plant breeding, the final selections thus being left for the localities in which the strains are to be grown. He pointed out that neither plan would necessarily have to be followed to the exclusion of the other, but that it would be well to decide at the outset of the program which plan should receive the greater emphasis. The first plan of practical breeding in one central location has limitations, however, since the environmental factors influencing the ultimate selection cannot be standardized for the region. Colby cited facts which would indicate that the twelve northeastern States are possibly more variable than any other region of similar size in the United States in regard to such factors as range of average seasonal temperature, extremes of summer and winter temperature, soil types, use and management of pastures, etc. Consequently he suggested that the second plan would seem to have greater possibilities, especially since many basic facts are not known concerning the breeding of pasture plants. In other parts of the world where pasture plants have been bred, e.g., Wales, New Zealand, and Svalöf, a practical program is carried on simultaneously with the fundamental research. In the Northeastern United States, however, where so many different types of environment are found and where so many diverse problems are presented to the different States, it

seems logical that a program for the central Laboratory would accomplish more through basic studies which might be applied over the entire Region than through practical breeding which probably would be limited in its application. Near the close of the general discussion of this topic a committee consisting of W. G. Colby, W. B. Kemp, and W. M. Myers was appointed to draw up a statement embodying the recommendations of the group. The committee's report, which was unanimously adopted when presented at the second morning meeting, follows:

> "The United States Regional Pasture Research Laboratory in cooperation with the twelve northeastern States is established to serve an area probably more diverse in soil and climate than any other agricultural area of equal size in this country. For instance, the average annual minimum temperature varies from -20°V. in Maine to 0°F. in Maryland. The average July temperature for Maine is 67° and for Maryland it is 75°. Soil types within this section vary from heavy clays to light sands.
>
> "In that no existing varieties of commercial farm crops are universally adapted in this area, it is not logical to expect that an agency in one location can develop strains of pasture plants adapted to localities other than those similar to its own. Therefore, it would seem that the Laboratory will best serve the region as a whole if its activities are directed to the investigation of the laws and principles underlying pasture improvement in this Region.
>
> "Accordingly, the work of the Laboratory should be confined chiefly to the fundamental rather than to the applied field. One of its functions, for example, should be the testing of different systems of breeding as they might be applied to several species, but actual breeding programs in most cases must be carried on in the localities where the strains are to be used."

2. <u>Characters to be sought in breeding pasture plants. Possible value of exploring existing variations within species now growing in pastures of the Northeast.</u>

Discussion Leader: H. B. Sprague, New Jersey Agr. Expt. Station.

Sprague outlined four principal objectives to guide the breeding of pasture plants: (1) more feed at low unit cost, (2) more even distribution of growth over the growing season, (3) quality of feed, e.g., protein or mineral content, absence of HCN, etc., and (4) palatability. Another

fundamental concept to be kept in mind is whether the soil should be improved for the plants or the plants be bred for poor soil. At present a middle course is perhaps the best since it is economically impossible to improve all pasture soils to a high level of fertility. Strains should at least be bred to utilize nutrients close to the surface, since soil treatments can be applied only on the surface of permanent pastures, and they are very slow to move in.

He suggested that emphasis might be placed on legumes in a breeding program because (1) they are a cheap source of N, (2) they have a high protein and mineral content (especially Ca and P), (3) they have a superior palatability, especially in that part of the summer when grasses are heading, and (4) some may have a greater drought resistance than grasses, dur probably to their deep root systems.

White clover is apparently the most important legume, considering the region as a whole. It exhibits a large range of forms, both in physiological and morphological traits. Although it is found over a wide range of soil types, it grows best where lime and minerals are abundant; this suggests a difference between plants in feeding power for nutrients. Its uniformity of growth depends on its ability to withstand low threshold temperatures in spring and fall as well as its ability to grow during the summer, when both moisture and temperature may be limiting factors. White clover would be benefited, however, by a deeper root system. A difference between plants in agressiveness is also observed; some plants tend to occupy the whole area and exclude grasses, while others are more tolerant of grasses. The later type may be better for some purposes, but a denser cover might better preserve a high moisture content and a lower soil temperature. The question of why white clover kills out at intervals is also of interest. This may be accounted for by the fact that it builds up an excess of N, which inhibits nodulation and proper growth. In this connection Virtanen has noted better

growth of both legumes and their associates where the legumes are not too dense. A variation is found also in the type of stolon; it is possible that a thick, fleshy stolon might be injured by livestock, whereas a thin tough one might stand trampling better. Other questions of interest are chemical content and its relation to palatability, disease resistance, HCN content and its relation to animal diseases, blooming, and seed production.

Alsike clover is supposed to be a perennial, but it does not behave as one in New Jersey. Greater longevity is needed as well as more prostrate growth forms. Its deep roots make it better adapted than white clover to light soils, which are often the predominant type in permanent pastures.

Mammoth red clover may be better adapted than medium red because of its greater feeding power, but it probably will never be useful in long leys.

Zigzag clover is a widely adapted species and should be particularly useful in pastures because of its underground stems. Before it could be widely used, however, its sterility and seed setting would have to be improved. It is very persistent and capable of withstanding vertical competition. Because of this, it could survive haying of pasture fields better than could white clover.

Bird's foot trefoil may be a useful pasture species, especially if low growing types, such as have been found in New York, prove to be of value.

Small yellow trefoil is a winter annual which may have a place if the growth of white clover cannot be extended farther into late fall and early spring. It grows well on the soils which are poor for white clover.

Lespedeza may be of value in some southern parts of the region where white clover does not grow well.

Improvement of grasses is important since they make up fifty to seventy-five percent of the total feed. Kentucky bluegrass is already abundant in the region, is high in nutrients, is relatively palatable, and grows early in the spring and late in the fall. It is poor in feeding power for soil nutrients, becomes dormant during summer, and is susceptible to certain

diseases, particularly leaf spot. Deeper root systems may be important.

Orchard grass is drought resistant, grows early in spring, continues through midsummer and late in the fall, is high in phosphorus and fairly deep rooted. It is no more unpalatable when it has reached the bloom stage of development than other grasses. Select for adapted pasture types.

Timothy is well adapted to the region and is very palatable. It is not adapted to poor soils and selection may improve this. Both orchard and timothy are excellent pasture grasses when used in mixtures.

Meadow fescue has all the good characteristics of timothy except seeding. It has greater longevity, lower crowns and greater drought resistance. Meadow fescue is susceptible to rust and other diseases. This grass is worthy of investigation.

Red top has perhaps the widest range of adaptability of any of the species with great variation within the species. It is a strong feeder for calcium and produces feed later in the season than Kentucky bluegrass or timothy. Types with underground and types with above ground stems occur. It is unpalatable and low in phosphorus and these characteristics should be improved.

Colonial bent is more palatable but less widely adapted than red top. A better spreading habit and greater disease resistance are needed.

Creeping bent may be valuable for wet pasture lands, particularly the leafy types.

Canada bluegrass is tolerant of poor dry uplands and also on water logged soils. It grows well during midsummer but is not very leafy or palatable. Breeding for greater palatability would make this an important species.

Red fescue is not palatable and is not tolerant to a very great range of temperatures. Its tolerance of poor soils makes it a worthwhile species for improvement.

In the discussion of grasses, Musser suggested that Lolium, tall oat grass and sweet vernal grass be added to the list of species to receive consideration.

In conclusion Sprague listed some general concepts to be kept in mind (1) The types selected must grow well in harmonious mixtures as well as have valuable characters in themselves, e.g., the value of legumes is shown by th fact that in New Jersey hay cost $13.00 per ton when legumes were included, but it cost $17.00 without legumes; (2) a uniform seasonal growth is important (a midsummer deficienty in permanent pastures is improved by legumes); (3) the problem of where to find the most desirable types needs consideratio since most important pasture plants in the northeast are introduced species. He suggested that a search at the centers of origin might be necessary, but at present it is probably better to start with the range that is here.

In the rather extended discussion that followed several interesting points were raised. Kemp questioned the direct relation between (1) adaptability to different mineral content of the soil, (2) mineral content of the plant, and (3) feeding power of the plant. He added that in Maryland black medick was capable of adjusting itself to either low or high clipping. Hollowell said the same was true of Ladino clover when growing with timothy. He noted, however, that it will not stand too close clipping in the summer. Its winter killing is not due only to cold temperature, since it survives in the northwestern United States. Colby questioned whether winter hardiness of Ladino was related to soil fertility. Hollowell added that moisture must be more important than temperature in determining white clover's persistence through the summer, since it lived in Missouri when the temperature was 109°, but the soil was wet.

3. Should attention be confined to the so-called permanent pasture plants?

Discussion Leader: H. B. Musser, Pennsylvania State Agr. Expt. Sta.

Musser discussed in some detail the value for temporary pastures of

red clover, alsike clover, zigzag clover, alfalfa, sweet clover, crimson clover, Lolium, millet, tall-oat grass, sweet-vernal grass, and Sudan grass. In connection with studies on these various species, the important question is whether one should spread out the work thinly over many species or concentrate on a few. Most persons present seemed to favor concentration of effort on a few species.

It was obvious that all parts of the Region could not be served by limiting the Laboratory work to the long-lived permanent pasture plants such as the bluegrasses and white clover. In addition there seemed to be real need for high-yielding, shorter-lived, rotational pasture plants such as timothy, orchard grass, ryegrass, and Ladino clover and for supplementary pasture plants such as Sudan grass and the millets.

4. Reproduction in the Poas. Apomixis, its occurrence and significance.

Discussion Leader: W. H. Brittingham, Regional Pasture Laboratory

Brittingham discussed the present status of our knowledge concerning apomixis and amphimixis in the bluegrasses. He also pointed out the relation between these methods of reproduction and the various breeding methods that might be used. In the discussion that followed it was generally agreed that these problems are of fundamental interest and should be worked upon at the Laboratory. A solution of the problems would be of great value in practical breeding.

5. Controlled pollination of pasture plants. Self-, Cross-, and male-sterility and their place in a breeding program.

Discussion Leader: S. S. Atwood, Regional Pasture Laboratory.

Atwood pointed out that since most of the plants which are important in permanent pastures in the northeast are naturally cross-pollinated, some breeding method based on controlled pollination such as is used with corn may be found to be most profitable. Inbreeding is complicated, however, by factors conditioning self-, cross-, and male-sterility. On the other hand

some sort of sterility may prove very useful in the final outcrossing of inbred lines. Those participating in the discussion agreed that a comparative study of breeding methods as applied to pasture plants should be undertaken.

Kemp suggested that mass selection may prove more useful in forage plants than in corn for the following reason: In the forage plants, the improvement is desired for the most part in vegetative characters; in corn it is desired more in seed characters. The natural selection which has been operating on every species may have tended to isolate the more favorable reproductive characters, which are not necessarily associated with the most desirable vegetative characters. Thus in corn, where improved seed characters are especially wanted, natural selection may have been operating to raise the level of production before man's artificial selection began. In the forage plants, however, mass selection may be able to raise the level of production considerably above that achieved by natural selection, since the production is determined here less by reproductive characters and more by vegetative characters, which may not have been favored by natural selection.

A majority of those present favored strain building (some form of mass selection) as a method of breeding, avoiding inbreeding as it is avoided by the Welsh and Canadian workers. Others felt that inbreeding followed by hybridization had a distinct place in the program. There seemed to be some difference of opinion on this point. Those who seemed to favor inbreeding recognized the value of mass selection as a method yielding quick and significant results. Nevertheless, they felt that inbreeding as a long-time program might eventually yield greater returns than would be possible by mass selection.

6. <u>Inbreeding and heterosis and their possible significance particularly with reference to clover and grass breeding.</u>

Discussion Leader: C. R. Burnham (by proxy) W. Va. Agr. Expt. Station.

After outlining the main steps in developing higher yielding hybrid

corn, Burnham (by proxy) discussed briefly the reasons for adopting such breeding methods with other cross-pollinated plants. He suggested that in corn it may never be possible to obtain a pure line with the yielding ability of the first generation superior hybrid between inbred lines. In other plants, such as white sweet clover, however, in which there is only thirty to forty percent of natural crossing, it has been possible to isolate pure lines, many of which appear as vigorous as the parent variety. The particular breeding method adopted therefore depends on the amount of natural cross-pollination within the species.

He suggested one other theoretical consideration which might apply in the case of pasture plants. The decrease in vigor in the F_2 generation is equal to 1/n times the increase in yield over the average yield of the parent inbreds, where n equals the number of inbred lines going into the hybrid. With a large number of good inbreds, the decrease in vigor might be so small that it would be offset by the advantage of maintaining the variety through natural pollination. In corn it has been difficult to get a multiple cross to a 16 x 16 stage for example, (when decrease would be 1/32) and still maintain the yield. There is also some lack of uniformity in such a cross, but this probably would be of some advantage in pasture plants.

7. Polyploidy: Existing polyploids among pasture species. Chromosome behavior and interspecific hybrids. Induced polyploidy as a means of extanding the range of hereditary variation.

Discussion Leader: W. M. Myers, Regional Pasture Laboratory.

Myers pointed out that many of the important pasture species for the northeastern Region, for example the bluegrasses, timothy, and white clover, are natural occurring polyploids. The breeding methods used for these species may be different than those applicable to ordinary diploids. Questions of fertility, inbreeding, etc., are necessarily complicated by the extra chromosomes, so that fundamental genetic and cytological studies on comparable diploid and ployploid species may be necessary to a complete

understanding of such problems. If the artificial induction of polyploids proves practical, it may be that work along this line will extend the existant range of variation and adaptation within a species. Another method of obtaining new forms depends on interspecific hybridization followed by chromosome doubling, or the hybridization of already existing autopolyploids.

8. <u>Methods of breeding pasture plants with reference to certain species.</u>

Discussion Leader: W. B. Kemp, Maryland Agr. Expt. Station.

Kemp discussed several problems which have presented themselves in his work:

(1) He observed that seed setting was poor in zigzag clover, meadow foxtail, and Bermuda grass. Since each of these species is really on the border of its range of adaptation in Maryland, he suggested that most of the existing plants may be closely related. Consequently, their cross-sterility may really be modified self-sterility.

Hollowell commented in this connection that in pollination between seven hundred plants of zigzag clover in Washington, D. C., there was just as much cross-incompatibility between closely related plants as there was between unrelated plants. The season at which the pollinations were made also affected the amount of sterility.

(2) After outlining his method of study with plant collections from pastures having different types of management, Kemp discussed the effect of this type of natural selection on the growth forms of Kentucky bluegrass and orchard grass. He concluded that plants with the shortest leaf sheath and the greatest angle between sheath and blad were naturally selected in closely grazed pastures. It was observed, however, that these plants with short sheaths generally had poor root systems. On continued examination, he found that prostrate plants tended to have better root systems, and that they also persisted under close grazing.

(3) The problem of midsummer drought, which is very important throughout the Northeastern Region, has been partly solved in Maryland with the

growing of Lespedeza; it is sown with rye, which is grazed in the spring, since the Lespedeza starts slowly. Kemp suggested that ryegrass might supply early and late grazing especially if locally adapted plants could be selected from pastures which are grazed the entire year (on March 18th they had already made one lawn-mower cutting on the Lolium plots).

(4) As a stop-gap in the regional breeding program until more refined methods can be worked out, he suggested that the Laboratory undertake a program of collecting plants of several species from many different regions. Under suitable isolation they would be allowed to intercross for two generations. The resulting seed would then be sent to various localities in the Region where it would be sown in small grazed plots. After several years of natural selection under different types of management in each locality, the plants would be allowed to grow up to seed, which would be harvested for local consumption.

9. Seed problems in connection with pasture improvement through the use of improved strains.

Discussion Leader: T. E. Odland, Rhode Island Experiment Station.

Odland pointed out that new problems of seed production may be expected in the forage plants where improved types are selected for their vegetative characters rather than for seed characters as in the case of wheat and corn. He said that the value of improved seed of pasture plants must be demonstrated to the farmers and that too small amounts should be released in the beginning. The seed must be grown by good seedsmen, and the best methods of growing, harvesting, threshing, etc., must be worked out. To show the type of problems that may be encountered, he cited some of their experiences with the certification of bent grass seed in Rhode Island. It is possible that the problems will be solved only through the development of special seed producing areas.

Musser pointed out that he obtained 1,000 pounds of seed per acre from chewings fescue when grown in cultivated rows.

Cardon mentioned the European studies on inducing pasture plants into seeding habits by a change in environment (fertilizing, etc.,); these resulted in a complete change of growth habit.

Just before final adjournment each State representative described briefly the work in breeding pasture plants under way at his particular station.